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(71) Applicant *(for all designated States except US)*: THE GOVERNMENT OF THE UNITED STATES OF AMERICA as represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; Office of Technology Transfer, National Institutes of Health, Suite 325, 6011 Executive Boulevard, Rockville, MD 20852 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: NAM, Jae-Hwan [KR/US]; 1001 Rockville Pike, #216, Rockville, MD 20852 (US). BUKH, Jens [DK/US]; 2018 Baltimore Road, #J42, Rockville, MD 20851 (US). EMERSON, Suzanne, U. [US/US]; 4517 Everett Street, Kensington, MD 20895 (US). PURCELL, Robert, H. [US/US]; 17517 White Ground Road, Boyds, MD 20841 (US).

(74) Agents: FEILER, William, S. et al.; Morgan & Finnegan, L.L.P., 345 Park Avenue, New York, NY 10154 (US).

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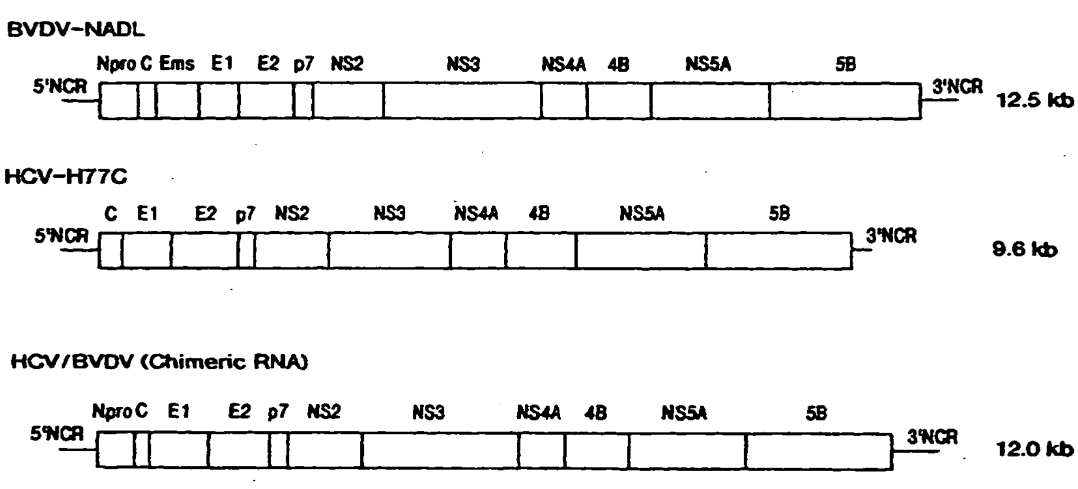
(54) Title: HCV/BVDV CHIMERIC GENOMES AND USES THEREOF



(57) Abstract: The present invention relates to molecular approaches to the production of nucleic acid sequences which comprise the genomes of chimeric hepatitis C virus-bovine viral diarrhea viruses (HCV-BVDV). The invention also relates to the use of these chimeric nucleic acid sequences to produce chimeric virions in cells and the use of these chimeric virions in HCV antibody neutralization assays, and for the development of vaccines and therapeutics for HCV.

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TITLE OF INVENTION

HCV/BVDV Chimeric Genomes and Uses Thereof

FIELD OF INVENTION

The present invention relates to molecular approaches to the production of nucleic acid sequences which comprise the genomes of chimeric hepatitis C virus-bovine viral diarrhea viruses (HCV-BVDV). The invention also relates to the use of these chimeric nucleic acid sequences to produce chimeric virions in cells and the use of these chimeric virions in HCV antibody neutralization assays, and for the development of vaccines and therapeutics for HCV.

Background Of Invention

Hepatitis C virus (HCV) has a positive-sense single-strand RNA genome and is a member of the genus Hepacivirus within the Flaviviridae family of viruses (Rice, 1996). As for all positive-stranded RNA viruses, the genome of HCV functions as mRNA from which all viral proteins necessary for propagation are translated.

The viral genome of HCV is approximately 9600 nucleotides (nts) in length and consists of a highly conserved 5′ untranslated region (UTR), a single long open reading frame (ORF) of approximately 9,000 nts and a complex 3′ UTR. The 5′ UTR contains an internal ribosomal entry site (Tsukiyama-Kohara et al., 1992; Honda et al., 1996). The 3′ UTR consists of a short variable region, a polypyrimidine tract of variable length and, at the 3′ end, a highly conserved region of approximately 100 nucleotides (Kolykhalov et al., 1996;

accounts for the majority of HCV infections but genotypes 2 and 3 each account for 5-15%.

At present, more than 80% of individuals infected with HCV become chronically infected and these chronically infected individuals have a relatively high risk of developing chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (Hoofnagle, 1997). The only effective therapy for chronic hepatitis C, interferon (IFN), alone or in combination with ribavirin, induces a sustained response in less than 50% of treated patients (Davis et al., 1998; McHutchinson et al., 1998). Consequently, HCV is currently the most common cause of end stage liver failure and the reason for about 30% of liver transplants performed in the U.S. (Hoofnagle, 1997). In addition, a number of recent studies suggested that the severity of liver disease and the outcome of therapy may be genotype-dependent (reviewed in Bukh et al., 1997). In particular, these studies suggested that infection with HCV genotype 1b was associated with more severe liver disease (Brechot, 1997) and a poorer response to IFN therapy (Fried and Hoofnagle, 1995). As a result of the inability to develop a universally effective therapy against HCV infection, it is estimated that there are still more than 25,000 new infections yearly in the U.S. (Alter 1997) Moreover, since there is no vaccine for HCV, HCV remains a serious public health problem.

Despite the intense interest in the development of vaccines and therapies for HCV, progress has been hindered by the absence of a useful cell culture system for laboratory study (2-7). For example, although the virus has been grown in some cell lines,

chimeric nucleic acid sequences to study the molecular properties of HCV indirectly in vitro.

The present invention also relates to the polypeptides encoded by the chimeric nucleic acid sequences of the invention or fragments thereof.

The invention also provides that the chimeric nucleic acid sequences and the chimeric viruses of the invention be supplied in the form of a kit, alone or in the form of a pharmaceutical composition.

DESCRIPTION OF FIGURES

Fig. 1. Genomic organization of BVDV, HCV and HCV/BVDV chimera. The BVDV and HCV are NADL (14, 21) and H77 strains (12), respectively. The complete BVDV-NADL genome consists of, in 5' to 3' order, 5'NCR (nucleotides 1-385), N^{pro} (nucleotides 386-889), Core (nucleotides 890-1195), E^{rns} (nucleotides 1196-1876), E1 (nucleotides 1877-2461), E2 (nucleotides 2462-3583), P7 and nonstructural genes (nucleotides 3584-12349) and 3'NCR (nucleotides 12352-12578).

Fig. 2. Strategy for the construction of chimeric cDNA, pHCV/BVDV-3, which has core, E1 and E2 of HCV in the backbone of BVDV. The fusion PCR products were cloned into pBV18-F2 after digestion with *SnaB* I and *Bsm* I. The fragments containing fusion PCR products were cloned into pSDMlu-3' after digestion with *Cla* I and *Dra* III.

Figures 3A-3H show the nucleotide and deduced amino acid sequences of the infectious HCV clone of genotype 1a.

Figures 4A-4H show the nucleotide and deduced amino acid sequences of the infectious clone of genotype

constructed. Such chimeras can be used to determine the relative importance of E1 or E2 for infection of cell lines. In another embodiment, HCV/BVDV chimeras in which one of the nonstructural genes of BVDV, such as NS3 RNA helicase, NS3 protease, or the NS5B RNA-dependent RNA polymerase are replaced by the corresponding non-structural genes of HCV may be constructed. Such chimeras would, for example, be useful in identifying inhibitors of viral enzyme activity which would be useful as antiviral agents.

In yet another embodiment, hypervariable region 1 (HVR1) from multiple HCV genotypes may be combined into one HCV/BVDV chimera. The only limit for constructing this type of chimera is that the viral genome must be able to be packaged. Alternatively, a chimera can be constructed which contain an HVR1 sequence from one HCV genotype. Such chimeras can be used as an inactivated multivalent vaccine or to screen for neutralizing antibodies to multiple HCV genotypes.

The HCV/BVDV chimeras of the invention may be constructed using any HCV and BVDV clones. However, in a preferred embodiment, the HCV clones are infectious HCV clones of genotype 1a (ATCC accession number PTA-157; Figures 3A-3F), 1b (ATCC accession number 209596; Figures 4A-4F) or 2a (ATCC accession number PTA-153; SEQ ID NOS:3-4) and the infectious BVDV clone pVVNADL are used.

In constructing the chimeric nucleic acid sequences of the invention, it is to be understood that the retention of the E^{rns} gene of BVDV in any chimeric is entirely optional. Thus, when it is stated that the HCV/BVDV chimeras could be constructed in which, for

the growing of animal cells *in vitro* and transfecting the cells with the chimeric nucleic acid of the invention, then determining if the cells show indicia of HCV infection. Such indicia include the detection of viral antigens in the cell, for example, by immunofluorescence procedures well known in the art; the detection of viral polypeptides by Western blotting using antibodies specific therefor; and the detection of newly transcribed viral RNA within the cells via methods such as RT-PCR. The presence of live, infectious virus particles following such tests may also be shown by injection of cell culture medium or cell lysates into healthy, susceptible animals, with subsequent exhibition of the signs and symptoms of HCV infection. Alternatively, the presence of live, infectious virus particles following such tests may also be shown by serial passaging the chimeric virus in cells.

Suitable cells or cell lines for culturing the chimeric viruses of the invention include, but are not limited to, EBTr(A) and Huh7.

Preferably, transfection of cells with the chimeric sequences is carried out in the presence of helper BVDV which is preferably of a noncytopathogenic strain. In one embodiment, the cell lines to be infected may already contain a helper BVDV. Such cells include, but are not limited to, EBTr(A). Alternatively, the cell lines to be transfected may be infected with a helper BVDV prior to, or concurrent with, transfection with the chimeric sequences of the invention.

The present invention also relates to polypeptides encoded by the chimeric nucleic acid

can then be used to immunize chimpanzees to determine whether the antibodies are protective. Alternatively, cells infected with the chimeric viruses of the invention may be passaged in cell culture to produce attenuated viruses which can be tested as candidate live vaccines. In assaying the ability of the chimeric viruses of the invention to infect mammals one can assay sera or liver of the infected mammal by RT-PCR to determine viral titer. In addition, the virulence phenotype of the virus produced by transfection of mammals with the sequences of the invention can be monitored by methods known in the art such as measurement of liver enzyme levels (alanine aminotransferase (ALT) or isocitrate dehydrogenase (ICD)) or by histopathology of liver biopsies.

Alternatively, mutations may be introduced into the HCV portion of the HCV/BVDV chimeras of the invention in order to enable the production of virions in cell cultures which could then be tested in vivo for improved vaccine properties.

In another embodiment, multiple chimeras containing HCV structural genes (or fragments thereof, such as the HVR1) from multiple genotypes can be administered to generate multivalent vaccines.

When used as a vaccine, the chimeric virions can be administered alone or in a suitable diluent, including, but not limited to, water, saline, or some type of buffered medium. The vaccine according to the present invention may be administered to an animal, especially a mammal, and most especially a human, by a variety of routes, including, but not limited to, intradermally, intramuscularly, subcutaneously, or in

serum free of BVDV and antibodies to BVDV (Boyt Veternary, Neoshoe, MO) was used. All cells were incubated at 37°C in 5% CO_2.

Table 1

List of Cell Lines

Cell	Origin	Medium
EBTr(A)	Embryonic bovine trachea	10% FBS/MEM
BT	Bovine turbinate	10% horse serum/MEM
MDBK	Bovine kidney	10% horse serum/MEM
EBTr (B)	Embryonic bovine trachea	10% FBS/MEM
Huh 7	human hepatoma	10% FBS/DMEM F12

Antibodies

H79: plasma from patient H obtained in the chronic phase two years after the onset of HCV infection (11); CH1530: serum pool from chimpanzee 1530, obtained in the chronic phase one to two years after the onset of HCV infection. Chimpanzee 1530 became infected with HCV following intrahepatic transfection with pCV-H77C (Yanagi 1997); LMF86 and LMF87: anti-HVR1 (Farci 1996), rabbit anti-peptide sera; Mab NS: anti-BVDV NS3 murine monoclonal antibody kindly provided by Dr. E. Dubovi (Cornell University, Ithaca, NY).

Construction of HCV/BVDV chimeric clone

The C, E1 and E2 genes originating from an infectious clone of the H77 strain of HCV (pCV-H77C, ref. Yanagi 1997), and the backbone originating from two subgenomic plasmids (pBV18-F2 and pSDMlu-3'), used by Vassilev et al. (Vassilev 1997) to generate the infectious clone of the NADL strain of BVDV (pVVNADL), were used to construct the chimeric cDNA clone pHCV-BVDV-3 (ATCC deposit Number PTA-158). The chimeric clone includes sequences corresponding to nucleotides

HCV/BVDV sequence of the final preparation was determined using standard procedures and about 90 specific sense and antisense primers. Clone pHCV/BVDV-3 was apparently stable since the digestion pattern was as expected following retransformation. The complete sequence differed slightly from the published BVDV sequence of the NADL strain (21), but encoded an intact polyprotein.

Table 2

Oligonucleotides used for PCR amplification

Name	Sequences (5' - 3')	Underline
N-C/H77/S	CAAGTTGCAGCACGAATCCTAAACCTCAAAGAA	N OF BVDV-NADL
Mlul/NADL/S	CACGCGTATCGATGAATTCG	Mlu i
B2-P7/NADL/S	AGCGGAGGCGATTCAGTATGGATCAGGGGAAGTG	E2 OF HCV
E2-P7/H77/R	ATACTGAATCGCCTCCGCTTGGGATATGAG	P7 OF BVDV-NADL
N-C/NADL/R	AGGATTCGTGCTGCAACTTGTGACCCATAGAGGG CAGTC	Core OF HCV
BanI/NADL/R	TACCAGGCTGAGAATGCACTGTAAC	Bsm I
2937S-HCBV	CCTTGTCCACCGGCCTCATCCACCTCCACC	
1353S-NADL	CAATTCATGGTATGATGGATGC	
1419S-NADL	AGTGGAACAAGCATGGTTGGTG	
2335-NADL	CCACGTGGACGAGGGCATGCC	
3342R-NADL	CCTGAATCGGCCTTTACCACATCCCCAATC	
1623R-NADL	TTCTTTCCTTTCTTGCAACCTGT	
1590R-NADL	GGGCTATCTCTAGCTTGTGTTAC	
389R-NADL	CCATGTGCCATGTACAGCAGAG	

Transfection of cell lines with transcribed RNA

The plasmid pHCV/BVDV-3 was linearized with SacII (NEB) and treated with T4 DNA polymerase (GIBCO/BRL) to remove the resulting 3' overhang. A truncated form of pHCV/BVDV-3, generated by digestion with HindIII, was used as a negative control. Two micrograms of DNA were transcribed at 37°C for 2 hrs in a 100 µl reaction volume containing 50 U of T7 RNA polymerase (Promega), 10 mM DTT (Promega), 120 U of Rnasin (Promega) and 1 mM rNTPs (GIBCO/BRL). Five microliters of the final reaction mixture was analyzed

with phosphate buffered saline (PBS) for 10 min. Thereafter, cells were incubated for 20-60 min at 37°C with primary antibodies diluted in 10% bovine serum albumin (BSA) in PBS. As primary antibodies we used an anti-HCV human plasma sample (H79, 1:100 dilution), an anti-HCV chimpanzee serum (CH1530, 1:100 dilution) and an anti-BVDV NS3 monoclonal antibody (Mab-NS, 1:10 dilution). After washing with PBS for 15 min, cells were incubated for 20-40 min at 37°C with secondary antibodies; fluorescein-isothiocyanate (FITC)-conjugated goat anti-human antibody (SIGMA) for H79 and CH1530, and rhodamine-conjugated anti-mouse antibody (PIERCE) for anti-BVDV NS3. For double staining, H79 or CH1530 anti-HCV antibody was mixed with the anti-BVDV NS3 monoclonal antibody and incubated on fixed cells as above, followed by washing and incubation with a mixture of both secondary antibodies. After washing, slides were mounted and examined by fluorescence microscopy (Zeiss).

Determination of sucrose
gradient density of recovered viruses

A T150 flask of EBTr(A) cells was inoculated with virus stock. At days 9 and 13, respectively, supernatant was harvested. A total of 70 ml of supernatant was layered over 20% sucrose in TN buffer [50mM Tris and 100mM NaCl (pH 7.4)] and centrifuged at 28,000 rpm in an SW28 swinging bucket rotor (Beckman) for 19 hrs at 4°C. The pellet was resuspended in 100 µl of TN buffer. For sucrose equilibrium gradient centrifugation, the resuspended pellet was layered onto a 20-60% (wt/wt) sucrose gradient in TN buffer and centrifuged at 36,000 rpm in an SW40 swinging bucket

incubated with ECL Western blotting detection reagent (Amersham) and exposed to film.

Detection of chimeric genomic RNA by RT-PCR assays

Total RNA was extracted with the TRIzol reagent from 10 or 100 µl of cell suspension, supernatant or material from the sucrose gradient. The RNA pellet was resuspended in 10 mM dithiothreitol (DTT) containing 5% (vol/vol) of RNAsin (20-40 U/µl) (Promega). The RT was performed with avian myeloblastosis virus reverse transcriptase (Promega) and the external anti-sense primer (see below) and PCR was performed with AmpliTaq Gold DNA polymerase (Perkin Elmer) as described (Bukh, 1998a). Specificity was confirmed by sequence analysis of selected DNA products. Each set of experiments included a low titer positive control sample and appropriate negative controls. HCV/BVDV chimeric genomes were detected in one round of PCR with the primers 2937S-HCBV and 3342R-HCBV (Table 2). The structural region of BVDV was detected in an RT-nested PCR with external primers 1353S-NADL and 1623R-NADL and internal primers 1419S-NADL and 1590R-NADL (Table 2). These primers were conserved among all known BVDV strains. Finally, the 5' UTR sequence of BVDV was detected by using universal primers that detect both HCV and BVDV (Bukh 1992, Yanagi 1996), as well as universally conserved BVDV primers (233S-NADL and 389R-NADL). The genome equivalent (GE) titer of HCV, BVDV and HCV/BVDV in positive samples was determined by RT-nested PCR on 10-fold serial dilutions of the extracted RNA (Bukh 1998a). One GE was defined as the number of genomes present in the highest dilution

the Vectastain Elite kit (Vector Laboratories, Burlingame, CA) for peroxidase staining per the manufacturer's directions. The peroxidase substrate kit was Vector VIP (Vector Laboratory). Color development was stopped by washing the slide with water followed by air drying. Foci were counted with the aid of a dissecting microscope.

Focus neutralization assay

The assay was performed exactly as for the focus assay except the 200 µl inoculum consisted of 100 µl of chimeric virus diluted in 10% DMEM, 20 µl undiluted test or control serum, and 80 µl 10% DMEM. Each 200 µl sample was incubated at 4° C in ice overnight prior to inoculation of cells. Sera included fetal calf serum (Boyt) and rabbit pre-immune serum as negative controls, hyperimmune rabbit antisera raised to peptides spanning the HVR1 region of the H27 strain of HCV (Farci, 1996), and goat anti-BVDV (VMRD Pullman, WA) prepared without azide. All sera had been heat-inactivated at 56° C for 30 minutes.

Immunofluoresence neutralization assay in Huh7 cells

Two hundred microliters of chimeric virus was mixed with 20 ul of serum or plasma, incubated on ice overnight and added to one well of a four-well chamber slide. After 2 hours at 30°C, 1 ml of agarose overlay was added as for the focus assay. Four days later, slides were fixed and stained as for immunofluoresence microscopy and stained cells were manually counted by scanning the entire well using a Zeis microscope and the 40X objective.

a particle containing significant amounts of HCV proteins.

Although the proportion of cells producing HCV proteins increased in EBTr(A) cells, it remained low in the MDBK, BT, and EBTr(B) cell lines, suggesting that the virus was not spreading in these cells. In order to determine if these cells were making infectious virus, a homologous transmission was attempted by removing supernatant from each transfected culture and adding it to a new culture of the same cell line. The only successful transmission was from the transfected EBTr(A) cells to naive EBTr(A) cells (Table 3). Therefore, although the chimeric virus genome could replicate in all four cell lines and produced HCV proteins, only in the EBTr(A) cells was virion morphogenesis coupled with availability of a receptor conducive to infection.

Table 3

Homologous passage and heterologous passage

	Transfection	Homologous passage	Heterologous passage
EBTr(A)	+	+	
EBTr (B)	+	−	+
BT	+	−	+
MDBK	+	−	+

: Supernatants from transfected cells were passed onto new cells of the same type.
: Supernatants from transfected EBTr(A) cells were passed to indicated cells.

Two heterologous transmission experiments were performed to determine if the three other cell lines released infectious particles. In the first experiment, supernatant from transfected MDBK cells was inoculated onto the EBTr(A) cells. Immunofluorescence microscopy

RT-PCR primers designed to amplify known BVDV strains were able to amplify a cDNA fragment from uninoculated EBTr(A) cultures (titer: 10^6 GE/ml). The sequence of the cDNA was determined and found to match that of the CP-7 strain of BVDV (18).

Based on the data that only EBTr cells harboring BVDV were able to produce infectious particles containing the chimeric genome, it was hypothesized that the endogenous virus was serving as a helper virus, possibly by providing BVDV structural proteins. In order to determine if the infectious chimeric particles contained BVDV glycoproteins, a focus assay was developed in which cells expressing the chimeric genome were identified by their reactivity with CH1530 anti-HCV serum. An infectivity titer of 10^5 chimeric viruses/ml was obtained for passage 10 virus, which had an RT-PCR titer of 10^8 to 10^9 GE/ml. Chimeric virus produced in EBTr(A) cells was examined for its susceptibility to neutralization by anti-serum to BVDV as compared to neutralization by anti-sera raised against the hypervariable region 1 (HVR1) of the same HCV strain as was in the chimera. Dilutions of chimeric virus were incubated overnight with anti-BVDV, anti-HCV or control sera and the number of infectious particles remaining was determined by the focus assay (Table 4). The number of foci in the rabbit and bovine serum controls decreased in parallel with the dilution factor, indicating that the assay was linear and reliable. The anti-HCV sera did not neutralize the chimera. In contrast, anti-BVDV eliminated all foci at each dilution, suggesting that each and every infectious particle contained BVDV glycoproteins and that they were

into these cells might be totally independent of HCV glycoprotein. Thirdly, the HCV E2 glycoprotein might not have folded properly to function or to be recognized by the antibody. The question of the neutralizing potential of the anti-HVR1 serum cannot be answered at this time. By an immunofluoresence microscopy assay, the anti-HVR1 serum had titers of 1:1600 and 1:3200 for rabbits LMF86 and LMF87 respectively but the antibody detected by this assay is not necessarily neutralizing antibody. The functionality of the HCV glycoproteins would best be proved by infecting cells which are not susceptible to infection by BVDV due to an absence of the BVDV receptor. Huh 7 cells were chosen as an experimental system to test for functional HCV glycoproteins because they are a human cell line which grows well and is of hepatocyte origin. Attempts to infect Huh 7 cells with the endogenous BVDV virus of the EBTr(A) cell line were not successful, suggesting either that the receptor for BVDV was absent or that the BVDV genome was unable to replicate in these cells. Attempts to infect the Huh 7 cells with the chimera were more successful. Four days after incubation with 2 X 10^4 EBTr(A) tissue culture infectious doses (TCID) of the chimera, Huh 7 cells could be stained with antibody to NS3 as well as with antibody to HCV. Quantification of the number of infected cells indicated that the inoculum contained 10^3 TCID /ml for Huh 7 as compared to 10^5/ml for EBTr(A) cells. Although the cells could be infected, the virus did not spread, suggesting that in Huh 7 cells , as in the MDBK and BT cells, virions either were not assembled or were not released from cells. Most likely, the CP-7 virus could not provide the

antibody but at a lower titer. Since the DNA vaccine expressed only the E2 glycoprotein, this protein must be involved in binding to Huh 7 cells. The plasma from chimp 1530 contained antibodies to the HCV envelope proteins as measured by ELISA or immunofluoresence microscopy but apparently, these were not neutralizing antibodies. Chimpanzee 1494 did not have demonstrable antiodies against the HCV glycoproteins so its failure to neutralize was not unexpected. Therefore, the chimera should be very useful for screening samples for neutralizing antibodies and discriminating between those that neutralize as compared to those that just bind.

Table 5

Neutralization of chimeric virus growth in Huh 7 cells[1]

	Number of foci[2]		
Virus dilution	Fetal Calf Serum (Boyt)	Anti-HCV HVR1	Anti-BVDV
Undiluted	191	298	0
Dilution (1:10)	23	43	0

1. Huh 7 cells were used for infection but the virus had been grown in EBTr (A) cells.

2. Foci stained with chimp 1530 anti-HCV and visualized by immunofluoresence microscopy.

glycoproteins were synthesized, it would be feasible to test purified chimeric virions as a candidate inactivated vaccine. Purified chimeric virions can be tested first in mice and if antibody to HCV is produced, the virions will be tested in chimpanzees to determine if the candidate vaccine is efficacious. The fact that virions grown in EBTr(A) cells were able to infect Huh 7 cells and were neutralized by some anti-HCV positive plasmas (Table 6) suggests that such chimeric viruses could be used to screen for neutralizing antibodies to HCV as well as to screen other cell lines for HCV receptors. The infectivity of the chimera proves the principle that HCV-BVDV chimeras can serve as a useful tool for studying the molecular biology of HCV. The glycoprotein genes from the five other genotypes of HCV can be similarly inserted into the BVDV backbone in order to provide an assay for antibodies to each genotype. Additional chimeras are being constructed in which the core protein of BVDV is included so that only the glycoproteins of HCV are introduced. If BVDV core is critical for encapsidation of the RNA, it may be possible to generate chimeric viruses in the absence of helper. It will also be revealing to determine if the HCV contribution to the chimera can be localized to either E1 or E2 alone. Such a chimera will be tested for its ability to infect EBTr(A) and Huh 7 cells. These studies will help determine the relative importance of E1 and E2 for infection of Huh 7 cells and may define any association with the BVDV glycoproteins. In addition, chimeras in which the BVDV nonstructural genes such as p7 or NS4B or NS5A are replaced with the corresponding genes of HCV may also be generated to

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has been replaced by the non-structural region of a hepatitis C virus genome.

8. The nucleic acid molecule of claim 7, wherein at least one gene from the non-structural region of the BVDV genome has been replaced by the corresponding gene from the non-structural region of a hepatitis C virus genome.

9. A DNA construct comprising the nucleic acid molecule of claims 1, 2 or 7.

10. An RNA transcript of the DNA construct of claim 9.

11. A polypeptide encoded by the nucleic acid molecule according to claim 1.

12. A polypeptide encoded by the nucleic acid molecule according to claim 2.

13. The polypeptide according to claim 12, wherein said polypeptide is selected from the group consisting of E1, E2 or C.

14. A host cell transfected with the DNA construct of claim 9.

15. A host cell transfected with the RNA transcript of claim 10.

16. A chimeric HCV-BVDV virus produced by transfecting a host cell with the DNA construct of claim 9.

17. A chimeric HCV-BVDV virus produced by transfecting a host cell with the RNA transcript of claim 10.

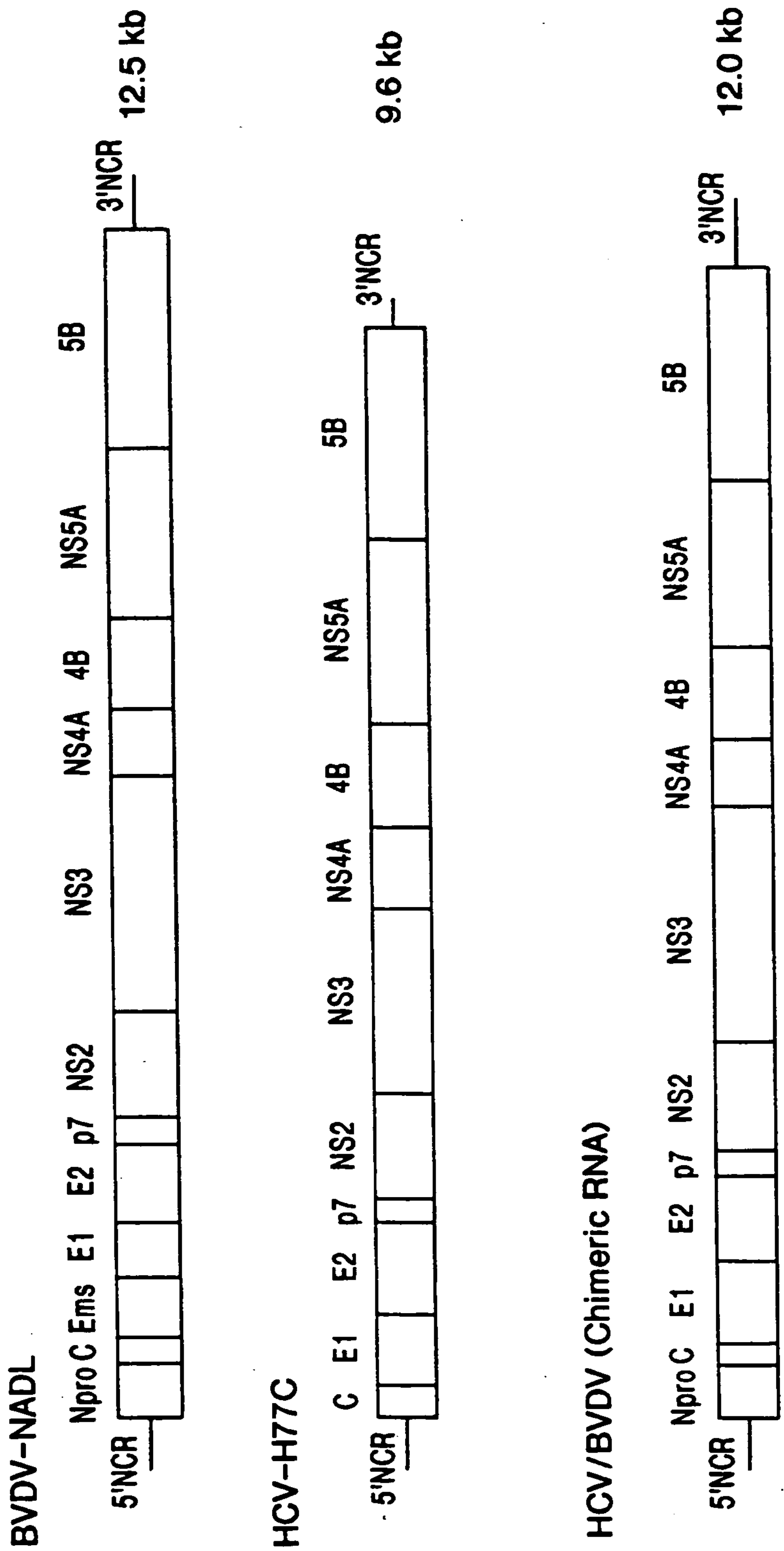
BVDV-NADL
5'NCR
Npro C Ems E1 E2 p7 NS2 NS3 NS4A 4B NS5A 5B
3'NCR
12.5 kb
HCV-H77C
5'NCR
C E1 E2 p7 NS2 NS3 NS4A 4B NS5A 5B
3'NCR
9.6 kb
HCV/BVDV (Chimeric RNA)
5'NCR
Npro C E1 E2 p7 NS2 NS3 NS4A 4B NS5A 5B
3'NCR
12.0 kb

FIG. 1

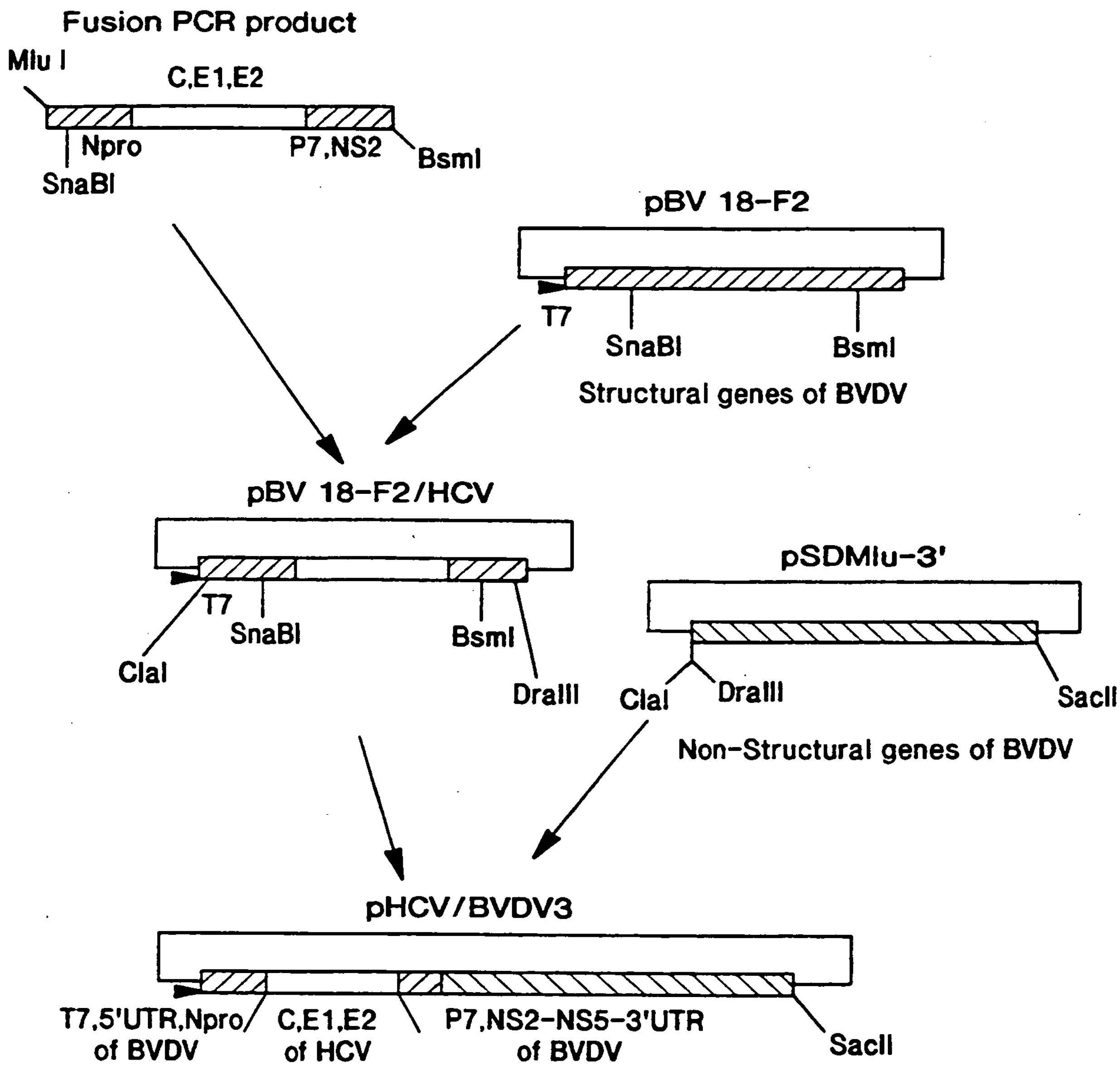
Fusion PCR product
Mlu I
C,E1,E2
Npro
P7,NS2
BsmI
SnaBI
pBV 18-F2
T7
SnaBI
BsmI
Structural genes of BVDV
pBV 18-F2/HCV
T7
SnaBI
BsmI
ClaI
DraIII
pSDMlu-3'
ClaI
DraIII
SacII
Non-Structural genes of BVDV
pHCV/BVDV3
T7,5'UTR,Npro of BVDV
C,E1,E2 of HCV
P7,NS2-NS5-3'UTR of BVDV
SacII

FIG. 2

H77C

```
        10         20         30         40         50
1234567890 1234567890 1234567890 1234567890 1234567890
GCCAGCCCCC TGATGGGGGC GACACTCCAC CATGAATCAC TCCCCTGTGA    50
GGAACTACTG TCTTCACGCA GAAAGCGTCT AGCCATGGCG TTAGTATGAG   100
TGTCGTGCAG CCTCCAGGAC CCCCCCTCCC GGGAGAGCCA TAGTGGTCTG   150
CGGAACCGGT GAGTACACCG GAATTGCCAG GACGACCGGG TCCTTTCTTG   200
GATAAACCCG CTCAATGCCT GGAGATTTGG GCGTGCCCCC GCAAGACTGC   250
TAGCCGAGTA GTGTTGGGTC GCGAAAGGCC TTGTGGTACT GCCTGATAGG   300
GTGCTTGCGA GTGCCCCGGG AGGTCTCGTA GACCGTGCAC CATGAGCACG   350
AATCCTAAAC CTCAAAGAAA AACCAAACGT AACACCAACC GTCGCCCACA   400
GGACGTCAAG TTCCCGGGTG GCGGTCAGAT CGTTGGTGGA GTTTACTTGT   450
TGCCGCGCAG GGGCCCTAGA TTGGGTGTGC GCGCGACGAG GAAGACTTCC   500
GAGCGGTCGC AACCTCGAGG TAGACGTCAG CCTATCCCCA AGGCACGTCG   550
GCCCGAGGGC AGGACCTGGG CTCAGCCCGG GTACCCTTGG CCCCTCTATG   600
GCAATGAGGG TTGCGGGTGG GCGGGATGGC TCCTGTCTCC CCGTGGCTCT   650
CGGCCTAGCT GGGGCCCCAC AGACCCCCGG CGTAGGTCGC GCAATTTGGG   700
TAAGGTCATC GATACCCTTA CGTGCGGCTT CGCCGACCTC ATGGGGTACA   750
TACCGCTCGT CGGCGCCCCT CTTGGAGGCG CTGCCAGGGC CCTGGCGCAT   800
GGCGTCCGGG TTCTGGAAGA CGGCGTGAAC TATGCAACAG GGAACCTTCC   850
TGGTTGCTCT TTCTCTATCT TCCTTCTGGC CCTGCTCTCT TGCCTGACTG   900
TGCCCGCTTC AGCCTACCAA GTGCGCAATT CCTCGGGGCT TTACCATGTC   950
ACCAATGATT GCCCTAACTC GAGTATTGTG TACGAGGCGG CCGATGCCAT  1000
CCTGCACACT CCGGGGTGTG TCCCTTGCGT TCGCGAGGGT AACGCCTCGA  1050
GGTGTTGGGT GGCGGTGACC CCCACGGTGG CCACCAGGGA CGGCAAACTC  1100
CCCACAACGC AGCTTCGACG TCATATCGAT CTGCTTGTCG GGAGCGCCAC  1150
CCTCTGCTCG GCCCTCTACG TGGGGGACCT GTGCGGGTCT GTCTTTCTTG  1200
TTGGTCAACT GTTTACCTTC TCTCCCAGGC GCCACTGGAC GACGCAAGAC  1250
TGCAATTGTT CTATCTATCC CGGCCATATA ACGGGTCATC GCATGGCATG  1300
GGATATGATG ATGAACTGGT CCCCTACGGC ACGGTTGGTG GTAGCTCAGC  1350
TGCTCCGGAT CCCACAAGCC ATCATGGACA TGATCGCTGG TGCTCACTGG  1400
GGAGTCCTGG CGGGCATAGC GTATTTCTCC ATGGTGGGGA ACTGGGCGAA  1450
GGTCCTGGTA GTGCTGCTGC TATTTGCCGG CGTCGACGCG GAAACCCACG  1500
TCACCGGGGG AAATGCCGGC CGCACCACGG CTGGGCTTGT TGGTCTCCTT  1550
ACACCAGGCG CCAAGCAGAA CATCCAACTG ATCAACACCA ACGGCAGTTG  1600
GCACATCAAT AGCACGGCCT TGAATTGCAA TGAAAGCCTT AACACCGGCT  1650
GGTTAGCAGG GCTCTTCTAT CAACACAAAT TCAACTCTTC AGGCTGTCCT  1700
GAGAGGTTGG CCAGCTGCCG ACGCCTTACC GATTTTGCCC AGGGCTGGGG  1750
TCCTATCAGT TATGCCAACG GAAGCGGCCT CGACGAACGC CCCTACTGCT  1800
GGCACTACCC TCCAAGACCT TGTGGCATTG TGCCCGCAAA GAGCGTGTGT  1850
GGCCCGGTAT ATTGCTTCAC TCCCAGCCCC GTGGTGGTGG GAACGACCGA  1900
```

FIG. 3A

H77C

10 1234567890	20 1234567890	30 1234567890	40 1234567890	50 1234567890	
CAGGTCGGGC	GCGCCTACCT	ACAGCTGGGG	TGCAAATGAT	ACGGATGTCT	1950
TCGTCCTTAA	CAACACCAGG	CCACCGCTGG	GCAATTGGTT	CGGTTGTACC	2000
TGGATGAACT	CAACTGGATT	CACCAAAGTG	TGCGGAGCGC	CCCCTTGTGT	2050
CATCGGAGGG	GTGGGCAACA	ACACCTTGCT	CTGCCCCACT	GATTGCTTCC	2100
GCAAACATCC	GGAAGCCACA	TACTCTCGGT	GCGGCTCCGG	TCCCTGGATT	2150
ACACCCAGGT	GCATGGTCGA	CTACCCGTAT	AGGCTTTGGC	ACTATCCTTG	2200
TACCATCAAT	TACACCATAT	TCAAAGTCAG	GATGTACGTG	GGAGGGGTCG	2250
AGCACAGGCT	GGAAGCGGCC	TGCAACTGGA	CGCGGGGCGA	ACGCTGTGAT	2300
CTGGAAGACA	GGGACAGGTC	CGAGCTCAGC	CCGTTGCTGC	TGTCCACCAC	2350
ACAGTGGCAG	GTCCTTCCGT	GTTCTTTCAC	GACCCTGCCA	GCCTTGTCCA	2400
CCGGCCTCAT	CCACCTCCAC	CAGAACATTG	TGGACGTGCA	GTACTTGTAC	2450
GGGGTAGGGT	CAAGCATCGC	GTCCTGGGCC	ATTAAGTGGG	AGTACGTCGT	2500
TCTCCTGTTC	CTTCTGCTTG	CAGACGCGCG	CGTCTGCTCC	TGCTTGTGGA	2550
TGATGTTACT	CATATCCCAA	GCGGAGGCGG	CTTTGGAGAA	CCTCGTAATA	2600
CTCAATGCAG	CATCCCTGGC	CGGGACGCAC	GGTCTTGTGT	CCTTCCTCGT	2650
GTTCTTCTGC	TTTGCGTGGT	ATCTGAAGGG	TAGGTGGGTG	CCCGGAGCGG	2700
TCTACGCCCT	CTACGGGATG	TGGCCTCTCC	TCCTGCTCCT	GCTGGCGTTG	2750
CCTCAGCGGG	CATACGCACT	GGACACGGAG	GTGGCCGCGT	CGTGTGGCGG	2800
CGTTGTTCTT	GTCGGGTTAA	TGGCGCTGAC	TCTGTCGCCA	TATTACAAGC	2850
GCTATATCAG	CTGGTGCATG	TGGTGGCTTC	AGTATTTTCT	GACCAGAGTA	2900
GAAGCGCAAC	TGCACGTGTG	GGTTCCCCCC	CTCAACGTCC	GGGGGGGCCG	2950
CGATGCCGTC	ATCTTACTCA	TGTGTGTAGT	ACACCCGACC	CTGGTATTTG	3000
ACATCACCAA	ACTACTCCTG	GCCATCTTCG	GACCCCTTTG	GATTCTTCAA	3050
GCCAGTTTGC	TTAAAGTCCC	CTACTTCGTG	CGCGTTCAAG	GCCTTCTCCG	3100
GATCTGCGCG	CTAGCGCGGA	AGATAGCCGG	AGGTCATTAC	GTGCAAATGG	3150
CCATCATCAA	GTTAGGGGCG	CTTACTGGCA	CCTATGTGTA	TAACCATCTC	3200
ACCCCTCTTC	GAGACTGGGC	GCACAACGGC	CTGCGAGATC	TGGCCGTGGC	3250
TGTGGAACCA	GTCGTCTTCT	CCCGAATGGA	GACCAAGCTC	ATCACGTGGG	3300
GGGCAGATAC	CGCCGCGTGC	GGTGACATCA	TCAACGGCTT	GCCCGTCTCT	3350
GCCCGTAGGG	GCCAGGAGAT	ACTGCTTGGG	CCAGCCGACG	GAATGGTCTC	3400
CAAGGGGTGG	AGGTTGCTGG	CGCCCATCAC	GGCGTACGCC	CAGCAGACGA	3450
GAGGCCTCCT	AGGGTGTATA	ATCACCAGCC	TGACTGGCCG	GGACAAAAAC	3500
CAAGTGGAGG	GTGAGGTCCA	GATCGTGTCA	ACTGCTACCC	AAACCTTCCT	3550
GGCAACGTGC	ATCAATGGGG	TATGCTGGAC	TGTCTACCAC	GGGGCCGGAA	3600
CGAGGACCAT	CGCATCACCC	AAGGGTCCTG	TCATCCAGAT	GTATACCAAT	3650
GTGGACCAAG	ACCTTGTGGG	CTGGCCCGCT	CCTCAAGGTT	CCCGCTCATT	3700
GACACCCTGT	ACCTGCGGCT	CCTCGGACCT	TTACCTGGTC	ACGAGGCACG	3750
CCGATGTCAT	TCCCGTGCGC	CGGCGAGGTG	ATAGCAGGGG	TAGCCTGCTT	3800

FIG. 3B

H77C

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
TCGCCCCGGC	CCATTTCCTA	CTTGAAAGGC	TCCTCGGGGG	GTCCGCTGTT	3850
GTGCCCCGCG	GGACACGCCG	TGGGCCTATT	CAGGGCCGCG	GTGTGCACCC	3900
GTGGAGTGGC	TAAAGCGGTG	GACTTTATCC	CTGTGGAGAA	CCTAGGGACA	3950
ACCATGAGAT	CCCCGGTGTT	CACGGACAAC	TCCTCTCCAC	CAGCAGTGCC	4000
CCAGAGCTTC	CAGGTGGCCC	ACCTGCATGC	TCCCACCGGC	AGCGGTAAGA	4050
GCACCAAGGT	CCCGGCTGCG	TACGCAGCCC	AGGGCTACAA	GGTGTTGGTG	4100
CTCAACCCCT	CTGTTGCTGC	AACGCTGGGC	TTTGGTGCTT	ACATGTCCAA	4150
GGCCCATGGG	GTTGATCCTA	ATATCAGGAC	CGGGGTGAGA	ACAATTACCA	4200
CTGGCAGCCC	CATCACGTAC	TCCACCTACG	GCAAGTTCCT	TGCCGACGGC	4250
GGGTGCTCAG	GAGGTGCTTA	TGACATAATA	ATTTGTGACG	AGTGCCACTC	4300
CACGGATGCC	ACATCCATCT	TGGGCATCGG	CACTGTCCTT	GACCAAGCAG	4350
AGACTGCGGG	GGCGAGACTG	GTTGTGCTCG	CCACTGCTAC	CCCTCCGGGC	4400
TCCGTCACTG	TGTCCCATCC	TAACATCGAG	GAGGTTGCTC	TGTCCACCAC	4450
CGGAGAGATC	CCCTTTTACG	GCAAGGCTAT	CCCCCTCGAG	GTGATCAAGG	4500
GGGGAAGACA	TCTCATCTTC	TGCCACTCAA	AGAAGAAGTG	CGACGAGCTC	4550
GCCGCGAAGC	TGGTCGCATT	GGGCATCAAT	GCCGTGGCCT	ACTACCGCGG	4600
TCTTGACGTG	TCTGTCATCC	CGACCAGCGG	CGATGTTGTC	GTCGTGTCGA	4650
CCGATGCTCT	CATGACTGGC	TTTACCGGCG	ACTTCGACTC	TGTGATAGAC	4700
TGCAACACGT	GTGTCACTCA	GACAGTCGAT	TTCAGCCTTG	ACCCTACCTT	4750
TACCATTGAG	ACAACCACGC	TCCCCCAGGA	TGCTGTCTCC	AGGACTCAAC	4800
GCCGGGGCAG	GACTGGCAGG	GGGAAGCCAG	GCATCTATAG	ATTTGTGGCA	4850
CCGGGGGAGC	GCCCCTCCGG	CATGTTCGAC	TCGTCCGTCC	TCTGTGAGTG	4900
CTATGACGCG	GGCTGTGCTT	GGTATGAGCT	CACGCCCGCC	GAGACTACAG	4950
TTAGGCTACG	AGCGTACATG	AACACCCCGG	GGCTTCCCGT	GTGCCAGGAC	5000
CATCTTGAAT	TTTGGGAGGG	CGTCTTTACG	GGCCTCACTC	ATATAGATGC	5050
CCACTTTTTA	TCCCAGACAA	AGCAGAGTGG	GGAGAACTTT	CCTTACCTGG	5100
TAGCGTACCA	AGCCACCGTG	TGCGCTAGGG	CTCAAGCCCC	TCCCCCATCG	5150
TGGGACCAGA	TGTGGAAGTG	TTTGATCCGC	CTTAAACCCA	CCCTCCATGG	5200
GCCAACACCC	CTGCTATACA	GACTGGGCGC	TGTTCAGAAT	GAAGTCACCC	5250
TGACGCACCC	AATCACCAAA	TACATCATGA	CATGCATGTC	GGCCGACCTG	5300
GAGGTCGTCA	CGAGCACCTG	GGTGCTCGTT	GGCGGCGTCC	TGGCTGCTCT	5350
GGCCGCGTAT	TGCCTGTCAA	CAGGCTGCGT	GGTCATAGTG	GGCAGGATCG	5400
TCTTGTCCGG	GAAGCCGGCA	ATTATACCTG	ACAGGGAGGT	TCTCTACCAG	5450
GAGTTCGATG	AGATGGAAGA	GTGCTCTCAG	CACTTACCGT	ACATCGAGCA	5500
AGGGATGATG	CTCGCTGAGC	AGTTCAAGCA	GAAGGCCCTC	GGCCTCCTGC	5550
AGACCGCGTC	CCGCCATGCA	GAGGTTATCA	CCCCTGCTGT	CCAGACCAAC	5600
TGGCAGAAAC	TCGAGGTCTT	TTGGGCGAAG	CACATGTGGA	ATTTCATCAG	5650
TGGGATACAA	TACTTGGCGG	GCCTGTCAAC	GCTGCCTGGT	AACCCCGCCA	5700

FIG. 3C

H77C

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
TTGCTTCATT	GATGGCTTTT	ACAGCTGCCG	TCACCAGCCC	ACTAACCACT	5750
GGCCAAACCC	TCCTCTTCAA	CATATTGGGG	GGGTGGGTGG	CTGCCCAGCT	5800
CGCCGCCCCC	GGTGCCGCTA	CTGCCTTTGT	GGGTGCTGGC	CTAGCTGGCG	5850
CCGCCATCGG	CAGCGTTGGA	CTGGGGAAGG	TCCTCGTGGA	CATTCTTGCA	5900
GGGTATGGCG	CGGGCGTGGC	GGGAGCTCTT	GTAGCATTCA	AGATCATGAG	5950
CGGTGAGGTC	CCCTCCACGG	AGGACCTGGT	CAATCTGCTG	CCCGCCATCC	6000
TCTCGCCTGG	AGCCCTTGTA	GTCGGTGTGG	TCTGCGCAGC	AATACTGCGC	6050
CGGCACGTTG	GCCCGGGCGA	GGGGGCAGTG	CAATGGATGA	ACCGGCTAAT	6100
AGCCTTCGCC	TCCCGGGGGA	ACCATGTTTC	CCCCACGCAC	TACGTGCCGG	6150
AGAGCGATGC	AGCCGCCCGC	GTCACTGCCA	TACTCAGCAG	CCTCACTGTA	6200
ACCCAGCTCC	TGAGGCGACT	GCATCAGTGG	ATAAGCTCGG	AGTGTACCAC	6250
TCCATGCTCC	GGTTCCTGGC	TAAGGGACAT	CTGGGACTGG	ATATGCGAGG	6300
TGCTGAGCGA	CTTTAAGACC	TGGCTGAAAG	CCAAGCTCAT	GCCACAACTG	6350
CCTGGGATTC	CCTTTGTGTC	CTGCCAGCGC	GGGTATAGGG	GGGTCTGGCG	6400
AGGAGACGGC	ATTATGCACA	CTCGCTGCCA	CTGTGGAGCT	GAGATCACTG	6900
GACATGTCAA	AAACGGGACG	ATGAGGATCG	TCGGTCCTAG	GACCTGCAGG	6950
AACATGTGGA	GTGGGACGTT	CCCCATTAAC	GCCTACACCA	CGGGCCCCTG	6550
TACTCCCCTT	CCTGCGCCGA	ACTATAAGTT	CGCGCTGTGG	AGGGTGTCTG	6600
CAGAGGAATA	CGTGGAGATA	AGGCGGGTGG	GGGACTTCCA	CTACGTATCG	6650
GGTATGACTA	CTGACAATCT	TAAATGCCCG	TGCCAGATCC	CATCGCCCGA	6700
ATTTTTCACA	GAATTGGACG	GGGTGCGCCT	ACACAGGTTT	GCGCCCCCTT	6750
GCAAGCCCTT	GCTGCGGGAG	GAGGTATCAT	TCAGAGTAGG	ACTCCACGAG	6800
TACCCGGTGG	GGTCGCAATT	ACCTTGCGAG	CCCGAACCGG	ACGTAGCCGT	6850
GTTGACGTCC	ATGCTCACTG	ATCCCTCCCA	TATAACAGCA	GAGGCGGCCG	6900
GGAGAAGGTT	GGCGAGAGGG	TCACCCCCTT	CTATGGCCAG	CTCCTCGGCT	6950
AGCCAGCTGT	CCGCTCCATC	TCTCAAGGCA	ACTTGCACCG	CCAACCATGA	7000
CTCCCCTGAC	GCCGAGCTCA	TAGAGGCTAA	CCTCCTGTGG	AGGCAGGAGA	7050
TGGCGGGCAA	CATCACCAGG	GTTGAGTCAG	AGAACAAAGT	GGTGATTCTG	7100
GACTCCTTCG	ATCCGCTTGT	GGCAGAGGAG	GATGAGCGGG	AGGTCTCCGT	7150
ACCTGCAGAA	ATTCTGCGGA	AGTCTCGGAG	ATTCGCCCGG	GCCCTGCCCG	7200
TCTGGGCGCG	GCCGGACTAC	AACCCCCCGC	TAGTAGAGAC	GTGGAAAAAG	7250
CCTGACTACG	AACCACCTGT	GGTCCATGGC	TGCCCGCTAC	CACCTCCACG	7300
GTCCCCTCCT	GTGCCTCCGC	CTCGGAAAAA	GCGTACGGTG	GTCCTCACCG	7350
AATCAACCCT	ATCTACTGCC	TTGGCCGAGC	TTGCCACCAA	AAGTTTTGGC	7400
AGCTCCTCAA	CTTCCGGCAT	TACGGGCGAC	AATACGACAA	CATCCTCTGA	7450
GCCCGCCCCT	TCTGGCTGCC	CCCCCGACTC	CGACGTTGAG	TCCTATTCTT	7500
CCATGCCCCC	CCTGGAGGGG	GAGCCTGGGG	ATCCGGATCT	CAGCGACGGG	7550
TCATGGTCGA	CGGTCAGTAG	TGGGGCCGAC	ACGGAAGATG	TCGTGTGCTG	7600

FIG. 3D

H77C

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
CTCAATGTCT	TATTCCTGGA	CAGGCGCACT	CGTCACCCCG	TGCGCTGCGG	7650
AAGAACAAAA	ACTGCCCATC	AACGCACTGA	GCAACTCGTT	GCTACGCCAT	7700
CACAATCTGG	TGTATTCCAC	CACTTCACGC	AGTGCTTGCC	AAAGGCAGAA	7750
GAAAGTCACA	TTTGACAGAC	TGCAAGTTCT	GGACAGCCAT	TACCAGGACG	7800
TGCTCAAGGA	GGTCAAAGCA	GCGGCGTCAA	AAGTGAAGGC	TAACTTGCTA	7850
TCCGTAGAGG	AAGCTTGCAG	CCTGACGCCC	CCACATTCAG	CCAAATCCAA	7900
GTTTGGCTAT	GGGGCAAAAG	ACGTCCGTTG	CCATCCCAGA	AAGGCCGTAG	7950
CCCACATCAA	CTCCGTGTGG	AAAGACCTTC	TGGAAGACAG	TGTAACACCA	8000
ATAGACACTA	CCATCATGGC	CAAGAACGAG	GTTTTCTGCG	TTCAGCCTGA	8050
GAAGGGGGGT	CGTAAGCCAG	CTCGTCTCAT	CGTGTTCCCC	GACCTGGGCG	8100
TGCGCGTGTG	CGAGAAGATG	GCCCTGTACG	ACGTGGTTAG	CAAGCTCCCC	8150
CTGGCCGTGA	TGGGAAGCTC	CTACGGATTC	CAATACTCAC	CAGGACAGCG	8200
GGTTGAATTC	CTCGTGCAAG	CGTGGAAGTC	CAAGAAGACC	CCGATGGGGT	8250
TCTCGTATGA	TACCCGCTGT	TTTGACTCCA	CAGTCACTGA	GAGCGACATC	8300
CGTACGGAGG	AGGCAATTTA	CCAATGTTGT	GACCTGGACC	CCCAAGCCCG	8350
CGTGGCCATC	AAGTCCCTCA	CTGAGAGGCT	TTATGTTGGG	GGCCCTCTTA	8400
CCAATTCAAG	GGGGGAAAAC	TGCGGCTACC	GCAGGTGCCG	CGCGAGCGGC	8450
GTACTGACAA	CTAGCTGTGG	TAACACCCTC	ACTTGCTACA	TCAAGGCCCG	8500
GGCAGCCTGT	CGAGCCGCAG	GGCTCCAGGA	CTGCACCATG	CTCGTGTGTG	8550
GCGACGACTT	AGTCGTTATC	TGTGAAAGTG	CGGGGGTCCA	GGAGGACGCG	8600
GCGAGCCTGA	GAGCCTTCAC	GGAGGCTATG	ACCAGGTACT	CCGCCCCCCC	8650
CGGGGACCCC	CCACAACCAG	AATACGACTT	GGAGCTTATA	ACATCATGCT	8700
CCTCCAACGT	GTCAGTCGCC	CACGACGGCG	CTGGAAAGAG	GGTCTACTAC	8750
CTTACCCGTG	ACCCTACAAC	CCCCCTCGCG	AGAGCCGCGT	GGGAGACAGC	8800
AAGACACACT	CCAGTCAATT	CCTGGCTAGG	CAACATAATC	ATGTTTGCCC	8850
CCACACTGTG	GGCGAGGATG	ATACTGATGA	CCCATTTCTT	TAGCGTCCTC	8900
ATAGCCAGGG	ATCAGCTTGA	ACAGGCTCTT	AACTGTGAGA	TCTACGGAGC	8950
CTGCTACTCC	ATAGAACCAC	TGGATCTACC	TCCAATCATT	CAAAGACTCC	9000
ATGGCCTCAG	CGCATTTTCA	CTCCACAGTT	ACTCTCCAGG	TGAAATCAAT	9050
AGGGTGGCCG	CATGCCTCAG	AAAACTTGGG	GTCCCGCCCT	TGCGAGCTTG	9100
GAGACACCGG	GCCCCGAGCG	TCCGCGCTAG	GCTTCTGTCC	AGAGGAGGCA	9150
GGGCTGCCAT	ATGTGGCAAG	TACCTCTTCA	ACTGGGCAGT	AAGAACAAAG	9200
CTCAAACTCA	CTCCAATAGC	GGCCGCTGGC	CGGCTGGACT	TGTCCGGTTG	9250
GTTCACGGCT	GGCTACAGCG	GGGGAGACAT	TTATCACAGC	GTGTCTCATG	9300
CCCGGCCCCG	CTGGTTCTGG	TTTTGCCTAC	TCCTGCTCGC	TGCAGGGGTA	9350
GGCATCTACC	TCCTCCCCAA	CCGATGAAGG	TTGGGGTAAA	CACTCCGGCC	9400
TCTTAAGCCA	TTTCCTGTTT	TTTTTTTTTT	TTTTTTTTTT	TTTTTCTTTT	9450
TTTTTTTCTT	TCCTTTCCTT	CTTTTTTTCC	TTTCTTTTTC	CCTTCTTTAA	9500

FIG. 3E

H77C

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
TGGTGGCTCC	ATCTTAGCCC	TAGTCACGGC	TAGCTGTGAA	AGGTCCGTGA	9550
GCCGCATGAC	TGCAGAGAGT	GCTGATACTG	GCCTCTCTGC	AGATCATGT	9599

FIG. 3F

H77C

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
MSTNPKPQRK	TKRNTNRRPQ	DVKFPGGGQI	VGGVYLLPRR	GPRLGVRATR	50
KTSERSQPRG	RRQPIPKARR	PEGRTWAQPG	YPWPLYGNEG	CGWAGWLLSP	100
RGSRPSWGPT	DPRRRSRNLG	KVIDTLTCGF	ADLMGYIPLV	GAPLGGAARA	150
LAHGVRVLED	GVNYATGNLP	GCSFSIFLLA	LLSCLTVPAS	AYQVRNSSGL	200
YHVTNDCPNS	SIVYEAADAI	LHTPGCVPCV	REGNASRCWV	AVTPTVATRD	250
GKLPTTQLRR	HIDLLVGSAT	LCSALYVGDL	CGSVFLVGQL	FTFSPRRHWT	300
TQDCNCSIYP	GHITGHRMAW	DMMMNWSPTA	ALVVAQLLRI	PQAIMDMIAG	350
AHWGVLAGIA	YFSMVGNWAK	VLVVLLLFAG	VDAETHVTGG	NAGRTTAGLV	400
GLLTPGAKQN	IQLINTNGSW	HINSTALNCN	ESLNTGWLAG	LFYQHKFNSS	450
GCPERLASCR	RLTDFAQGWG	PISYANGSGL	DERPYCWHYP	PRPCGIVPAK	500
SVCGPVYCFT	PSPVVVGTTD	RSGAPTYSWG	ANDTDVFVLN	NTRPPLGNWF	550
GCTWMNSTGF	TKVCGAPPCV	IGGVGNNTLL	CPTDCFRKHP	EATYSRCGSG	600
PWITPRCMVD	YPYRLWHYPC	TINYTIFKVR	MYVGGVEHRL	EAACNWTRGE	650
RCDLEDRDRS	ELSPLLLSTT	QWQVLPCSFT	TLPALSTGLI	HLHQNIVDVQ	700
YLYGVGSSIA	SWAIKWEYVV	LLFLLLADAR	VCSCLWMMLL	ISQAEAALEN	750
LVILNAASLA	GTHGLVSFLV	FFCFAWYLKG	RWVPGAVYAL	YGMWPLLLLL	800
LALPQRAYAL	DTEVAASCGG	VVLVGLMALT	LSPYYKRYIS	WCMWWLQYFL	850
TRVEAQLHVW	VPPLNVRGGR	DAVILLMCVV	HPTLVFDITK	LLLAIFGPLW	900
ILQASLLKVP	YFVRVQGLLR	ICALARKIAG	GHYVQMAIIK	LGALTGTYVY	950
NHLTPLRDWA	HNGLRDLAVA	VEPVVFSRME	TKLITWGADT	AACGDIINGL	1000
PVSARRGQEI	LLGPADGMVS	KGWRLLAPIT	AYAQQTRGLL	GCIITSLTGR	1050
DKNQVEGEVQ	IVSTATQTFL	ATCINGVCWT	VYHGAGTRTI	ASPKGPVIQM	1100
YTNVDQDLVG	WPAPQGSRSL	TPCTCGSSDL	YLVTRHADVI	PVRRRGDSRG	1150
SLLSPRPISY	LKGSSGGPLL	CPAGHAVGLF	RAAVCTRGVA	KAVDFIPVEN	1200
LGTTMRSPVF	TDNSSPPAVP	QSFQVAHLHA	PTGSGKSTKV	PAAYAAQGYK	1250
VLVLNPSVAA	TLGFGAYMSK	AHGVDPNIRT	GVRTITTGSP	ITYSTYGKFL	1300
ADGGCSGGAY	DIIICDECHS	TDATSILGIG	TVLDQAETAG	ARLVVLATAT	1350
PPGSVIVSHP	NIEEVALSTT	GEIPFYGKAI	PLEVIKGGRH	LIFCHSKKKC	1400
DELAAKLVAL	GINAVAYYRG	LDVSVIPTSG	DVVVVSTDAL	MTGFTGDFDS	1450
VIDCNTCVTQ	TVDFSLDPTF	TIETTTLPQD	AVSRTQRRGR	TGRGKPGIYR	1500
FVAPGERPSG	MFDSSVLCEC	YDAGCAWYEL	TPAETTVRLR	AYMNTPGLPV	1550
CQDHLEFWEG	VFTGLTHIDA	HFLSQTKQSG	ENFPYLVAYQ	ATVCARAQAP	1600
PPSWDQMWKC	LIRLKPTLHG	PTPLLYRLGA	VQNEVTLTHP	ITKYIMTCMS	1650
ADLEVVTSTW	VLVGGVLAAL	AAYCLSTGCV	VIVGRIVLSG	KPAIIPDREV	1700
LYQEFDEMEE	CSQHLPYIEQ	GMMLAEQFKQ	KALGLLQTAS	RHAEVITPAV	1750
QTNWQKLEVF	WAKHMWNFIS	GIQYLAGLST	LPGNPAIASL	MAFTAAVTSP	1800
LTTGQTLLFN	ILGGWVAAQL	AAPGAATAFV	GAGLAGAAIG	SVGLGKVLVD	1850
ILAGYGAGVA	GALVAFKIMS	GEVPSTEDLV	NLLPAILSPG	ALVVGVVCAA	1900

FIG. 3G

SUBSTITUTE SHEET (RULE26)

H77C

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
ILRRHVGPGE	GAVQWMNRLI	AFASRGNHVS	PIHYVPESDA	AARVTAILSS	1950
LTVTQLLRRL	HQWISSECTT	PCSGSWLRDI	WDWICEVLSD	FKTWLKAKLM	2000
PQLPGIPFVS	CQRGYRGVWR	GDGIMHTRCH	CGAEITGHVK	NGTMRIVGPR	2050
TCRNMWSGTF	PINAYTTGPC	TPLPAPNYKF	ALWRVSAEEY	VETRRVGDFH	2100
YVSGMTTDNL	KCPCQIPSPE	FFTELDGVRL	HRFAPPCKPL	LREEVSFRVG	2150
LHEYPVGSQL	PCEPEPDVAV	LTSMLTDPSH	ITAEAAGRRL	ARGSPPSMAS	2200
SSASQLSAPS	LKATCTANHD	SPDAELIEAN	LLWRQEMGGN	ITRVESENKV	2250
VILDSFDPLV	AEEDEREVSV	PAEILRKSRR	FARALPVWAR	PDYNPPLVET	2300
WKKPDYEPPV	VHGCPLPPPR	SPPVPPPRKK	RTVVLTESTL	STALAELATK	2350
SFGSSSTSGI	TGDNTTTSSE	PAPSGCPPDS	DVESYSSMPP	LEGEPGDPDL	2400
SDGSWSTVSS	GADTEDVVCC	SMSYSWTGAL	VTPCAAEEQK	LPINALSNSL	2450
LRHHNLVYST	TSRSACQRQK	KVTFDRLQVL	DSHYQDVLKE	VKAAASKVKA	2500
NLLSVEEACS	LTPPHSAKSK	FGYGAKDVRC	HARKAVAHIN	SVWKDLLEDS	2550
VTPIDTTIMA	KNEVFCVQPE	KGGRKPARLI	VFPDLGVRVC	EKMALYDVVS	2600
KLPLAVMGSS	YGFQYSPGQR	VEFLVQAWKS	KKTPMGFSYD	TRCFDSTVTE	2650
SDIRTEEAIY	QCCDLDPQAR	VAIKSLTERL	YVGGPLTNSR	GENCGYRRCR	2700
ASGVLTTSCG	NTLTCYIKAR	AACRAAGLQD	CTMLVCGDDL	VVICESAGVQ	2750
EDAASLRAFT	EAMTRYSAPP	GDPPQPEYDL	ELITSCSSNV	SVAHDGAGKR	2800
VYYLTRDPTT	PLARAAWETA	RHTPVNSWLG	NIIMFAPTLW	ARMILMTHFF	2850
SVLIARDQLE	QALNCEIYGA	CYSIEPLDLP	PIIQRLHGLS	AFSLHSYSPG	2900
EINRVAACLR	KLGVPPLRAW	RHRARSVRAR	LLSRGGRAAI	CGKYLFNWAV	2950
RTKLKLTPIA	AAGRLDLSGW	FTAGYSGGDI	YHSVSHARPR	WFWFCLLLLA	3000
AGVGIYLLPN	R				3011

FIG. 3H

HC-J4

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
GCCAGCCCCC	TGATGGGGGC	GACACTCCAC	CATGAATCAC	TCCCCTGTGA	50
GGAACTACTG	TCTTCACGCA	GAAAGCGTCT	AGCCATGGCG	TTAGTATGAG	100
TGTCGTGCAG	CCTCCAGGAC	CCCCCCTCCC	GGGAGAGCCA	TAGTGGTCTG	150
CGGAACCGGT	GAGTACACCG	GAATTGCCAG	GACGACCGGG	TCCTTTCTTG	200
GATCAACCCG	CTCAATGCCT	GGAGATTTGG	GCGTGCCCCC	GCGAGACTGC	250
TAGCCGAGTA	GTGTTGGGTC	GCGAAAGGCC	TTGTGGTACT	GCCTGATAGG	300
GTGCTTGCGA	GTGCCCCGGG	AGGTCTCGTA	GACCGTGCAC	CATGAGCACG	350
AATCCTAAAC	CTCAAAGAAA	AACCAAACGT	AACACCAACC	GCCGCCCACA	400
GGACGTCAAG	TTCCCGGGCG	GTGGTCAGAT	CGTTGGTGGA	GTTTACCTGT	450
TGCCGCGCAG	GGGCCCCAGG	TTGGGTGTGC	GCGCGACTAG	GAAGGCTTCC	500
GAGCGGTCGC	AACCTCGTGG	AAGGCGACAA	CCTATCCCAA	AGGCTCGCCG	550
ACCCGAGGGC	AGGGCCTGGG	CTCAGCCCGG	GTACCCTTGG	CCCCTCTATG	600
GCAATGAGGG	CCTGGGGTGG	GCAGGATGGC	TCCTGTCACC	CCGCGGCTCC	650
CGGCCTAGTT	GGGGCCCCAC	GGACCCCCGG	CGTAGGTCGC	GTAACTTGGG	700
TAAGGTCATC	GATACCCTTA	CATGCGGCTT	CGCCGATCTC	ATGGGGTACA	750
TTCCGCTCGT	CGGCGCCCCC	CTAGGGGGCG	CTGCCAGGGC	CTTGGCACAC	800
GGTGTCCGGG	TTCTGGAGGA	CGGCGTGAAC	TATGCAACAG	GGAACTTGCC	850
CGGTTGCTCT	TTCTCTATCT	TCCTCTTGGC	TCTGCTGTCC	TGTTTGACCA	900
TCCCAGCTTC	CGCTTATGAA	GTGCGCAACG	TGTCCGGGAT	ATACCATGTC	950
ACGAACGACT	GCTCCAACTC	AAGCATTGTG	TATGAGGCAG	CGGACGTGAT	1000
CATGCATACT	CCCGGGTGCG	TGCCCTGTGT	TCAGGAGGGT	AACAGCTCCC	1050
GTTGCTGGGT	AGCGCTCACT	CCCACGCTCG	CGGCCAGGAA	TGCCAGCGTC	1100
CCCACTACGA	CAATACGACG	CCACGTCGAC	TTGCTCGTTG	GGACGGCTGC	1150
TTTCTGCTCC	GCTATGTACG	TGGGGGATCT	CTGCGGATCT	ATTTTCCTCG	1200
TCTCCCAGCT	GTTCACCTTC	TCGCCTCGCC	GGCATGAGAC	AGTGCAGGAC	1250
TGCAACTGCT	CAATCTATCC	CGGCCATGTA	TCAGGTCACC	GCATGGCTTG	1300
GGATATGATG	ATGAACTGGT	CACCTACAAC	AGCCCTAGTG	GTGTCGCAGT	1350
TGCTCCGGAT	CCCACAAGCT	GTCGTGGACA	TGGTGGCGGG	GGCCCACTGG	1400
GGAGTCCTGG	CGGGCCTTGC	CTACTATTCC	ATGGTAGGGA	ACTGGGCTAA	1450
GGTTCTGATT	GTGGCGCTAC	TCTTTGCCGG	CGTTGACGGG	GAGACCCACA	1500
CGACGGGGAG	GGTGGCCGGC	CACACCACCT	CCGGGTTCAC	GTCCCTTTTC	1550
TCATCTGGGG	CGTCTCAGAA	AATCCAGCTT	GTGAATACCA	ACGGCAGCTG	1600
GCACATCAAC	AGGACTGCCC	TAAATTGCAA	TGACTCCCTC	CAAACTGGGT	1650
TCTTTGCCGC	GCTGTTTTAC	GCACACAAGT	TCAACTCGTC	CGGGTGCCCG	1700
GAGCGCATGG	CCAGCTGCCG	CCCCATTGAC	TGGTTCGCCC	AGGGGTGGGG	1750
CCCCATCACC	TATACTAAGC	CTAACAGCTC	GGATCAGAGG	CCTTATTGCT	1800
GGCATTACGC	GCCTCGACCG	TGTGGTGTCG	TACCCGCGTC	GCAGGTGTGT	1850
GGTCCAGTGT	ATTGTTTCAC	CCCAAGCCCT	GTTGTGGTGG	GGACCACCGA	1900

FIG. 4A

HC-J4

10	20	30	40	50	
1234567890	1234567890	1234567890	1234567890	1234567890	
TCGTTCCGGT	GTCCCTACGT	ATAGCTGGGG	GGAGAATGAG	ACAGACGTGA	1950
TGCTCCTCAA	CAACACGCGT	CCGCCACAAG	GCAACTGGTT	CGGCTGTACA	2000
TGGATGAATA	GTACTGGGTT	CACTAAGACG	TGCGGAGGTC	CCCCGTGTAA	2050
CATCGGGGGG	GTCGGTAACC	GCACCTTGAT	CTGCCCCACG	GACTGCTTCC	2100
GGAAGCACCC	CGAGGCTACT	TACACAAAAT	GTGGCTCGGG	GCCCTGGTTG	2150
ACACCTAGGT	GCCTAGTAGA	CTACCCATAC	AGGCTTTGGC	ACTACCCCTG	2200
CACTCTCAAT	TTTTCCATCT	TTAAGGTTAG	GATGTATGTG	GGGGGCGTGG	2250
AGCACAGGCT	CAATGCCGCA	TGCAATTGGA	CTCGAGGAGA	GCGCTGTAAC	2300
TTGGAGGACA	GGGATAGGTC	AGAACTCAGC	CCGCTGCTGC	TGTCTACAAC	2350
AGAGTGGCAG	ATACTGCCCT	GTGCTTTCAC	CACCCTACCG	GCTTTATCCA	2400
CTGGTTTGAT	CCATCTCCAT	CAGAACATCG	TGGACGTGCA	ATACCTGTAC	2450
GGTGTAGGGT	CAGCGTTTGT	CTCCTTTGCA	ATCAAATGGG	AGTACATCCT	2500
GTTGCTTTTC	CTTCTCCTGG	CAGACGCGCG	CGTGTGTGCC	TGCTTGTGGA	2550
TGATGCTGCT	GATAGCCCAG	GCTGAGGCCG	CCTTAGAGAA	CTTGGTGGTC	2600
CTCAATGCGG	CGTCCGTGGC	CGGAGCGCAT	GGTATTCTCT	CCTTTCTTGT	2650
GTTCTTCTGC	GCCGCCTGGT	ACATTAAGGG	CAGGCTGGCT	CCTGGGGCGG	2700
CGTATGCTTT	TTATGGCGTA	TGGCCGCTGC	TCCTGCTCCT	ACTGGCGTTA	2750
CCACCACGAG	CTTACGCCTT	GGACCGGGAG	ATGGCTGCAT	CGTGCGGGGG	2800
TGCGGTTCTT	GTAGGTCTGG	TATTCTTGAC	CTTGTCACCA	TACTACAAAG	2850
TGTTTCTCAC	TAGGCTCATA	TGGTGGTTAC	AATACTTTAT	CACCAGAGCC	2900
GAGGCGCACA	TGCAAGTGTG	GGTCCCCCCC	CTCAACGTTC	GGGGAGGCCG	2950
CGATGCCATC	ATCCTCCTCA	CGTGTGCGGT	TCATCCAGAG	TTAATTTTTG	3000
ACATCACCAA	ACTCCTGCTC	GCCATACTCG	GCCCGCTCAT	GGTGCTCCAG	3050
GCTGGCATAA	CGAGAGTGCC	GTACTTCGTG	CGCGCTCAAG	GGCTCATTCG	3100
TGCATGCATG	TTAGTGCGAA	AAGTCGCCGG	GGGTCATTAT	GTCCAAATGG	3150
TCTTCATGAA	GCTGGCCGCG	CTGACAGGTA	CGTACGTTTA	TAACCATCTT	3200
ACCCCACTGC	GGGACTGGGC	CCACGCGGGC	CTACGAGACC	TTGCGGTGGC	3250
GGTAGAGCCC	GTCGTCTTCT	CCGCCATGGA	GACCAAGGTC	ATCACCTGGG	3300
GAGCAGACAC	CGCTGCGTGT	GGGGACATCA	TCTTGGGTCT	ACCCGTCTCC	3350
GCCCGAAGGG	GGAAGGAGAT	ATTTTTGGGA	CCGGCTGATA	GTCTCGAAGG	3400
GCAAGGGTGG	CGACTCCTTG	CGCCCATCAC	GGCCTACTCC	CAACAAACGC	3450
GGGGCGTACT	TGGTTGCATC	ATCACTAGCC	TCACAGGCCG	GGACAAGAAC	3500
CAGGTCGAAG	GGGAGGTTCA	AGTGGTTTCT	ACCGCAACAC	AATCTTTCCT	3550
GGCGACCTGC	ATCAACGGCG	TGTGCTGGAC	TGTCTACCAT	GGCGCTGGCT	3600
CGAAGACCCT	AGCCGGTCCA	AAAGGTCCAA	TCACCCAAAT	GTACACCAAT	3650
GTAGACCTGG	ACCTCGTCGG	CTGGCAGGCG	CCCCCCGGGG	CGCGCTCCAT	3700
GACACCATGC	AGCTGTGGCA	GCTCGGACCT	TTACTTGGTC	ACGAGACATG	3750
CTGATGTCAT	TCCGGTGCGC	CGGCGAGGCG	ACAGCAGGGG	AAGTCTACTC	3800

FIG. 4B

HC-J4

10 1234567890	20 1234567890	30 1234567890	40 1234567890	50 1234567890	
TCCCCCAGGC	CCGTCTCCTA	CCTGAAAGGC	TCCTCGGGTG	GTCCATTGCT	3850
TTGCCCTTCG	GGGCACGTCG	TGGGCGTCTT	CCGGGCTGCT	GTGTGCACCC	3900
GGGGGGTCGC	GAAGGCGGTG	GACTTCATAC	CCGTTGAGTC	TATGGAAACT	3950
ACCATGCGGT	CTCCGGTCTT	CACAGACAAC	TCAACCCCCC	CGGCTGTACC	4000
GCAGACATTC	CAAGTGGCAC	ATCTGCACGC	TCCTACTGGC	AGCGGCAAGA	4050
GCACCAAAGT	GCCGGCTGCG	TATGCAGCCC	AAGGGTACAA	GGTGCTCGTC	4100
CTGAACCCGT	CCGTTGCCGC	CACCTTAGGG	TTTGGGGCGT	ATATGTCCAA	4150
GGCACACGGT	ATCGACCCTA	ACATCAGAAC	TGGGGTAAGG	ACCATTACCA	4200
CGGGCGGCTC	CATTACGTAC	TCCACCTATG	GCAAGTTCCT	TGCCGACGGT	4250
GGCTGTTCTG	GGGGCGCCTA	TGACATCATA	ATATGTGATG	AGTGCCACTC	4300
AACTGACTCG	ACTACCATCT	TGGGCATCGG	CACAGTCCTG	GACCAAGCGG	4350
AGACGGCTGG	AGCGCGGCTC	GTCGTGCTCG	CCACCGCTAC	ACCTCCGGGA	4400
TCGGTTACCG	TGCCACACCC	CAATATCGAG	GAAATAGGCC	TGTCCAACAA	4450
TGGAGAGATC	CCCTTCTATG	GCAAAGCCAT	CCCCATTGAG	GCCATCAAGG	4500
GGGGGAGGCA	TCTCATTTTC	TGCCATTCCA	AGAAGAAATG	TGACGAGCTC	4550
GCCGCAAAGC	TGACAGGCCT	CGGACTGAAC	GCTGTAGCAT	ATTACCGGGG	4600
CCTTGATGTG	TCCGTCATAC	CGCCTATCGG	AGACGTCGTT	GTCGTGGCAA	4650
CAGACGCTCT	AATGACGGGT	TTCACCGGCG	ATTTTGACTC	AGTGATCGAC	4700
TGCAATACAT	GTGTCACCCA	GACAGTCGAC	TTCAGCTTGG	ATCCCACCTT	4750
CACCATTGAG	ACGACGACCG	TGCCCCAAGA	CGCGGTGTCG	CGCTCGCAAC	4800
GGCGAGGTAG	AACTGGCAGG	GGTAGGAGTG	GCATCTACAG	GTTTGTGACT	4850
CCAGGAGAAC	GGCCCTCGGG	CATGTTCGAT	TCTTCGGTCC	TGTGTGAGTG	4900
CTATGACGCG	GGCTGTGCTT	GGTATGAGCT	CACGCCCGCT	GAGACCTCGG	4950
TTAGGTTGCG	GGCTTACCTA	AATACACCAG	GGTTGCCCGT	CTGCCAGGAC	5000
CATCTGGAGT	TCTGGGAGAG	CGTCTTCACA	GGCCTCACCC	ACATAGATGC	5050
CCACTTCCTG	TCCCAGACTA	AACAGGCAGG	AGACAACTTT	CCTTACCTGG	5100
TGGCATATCA	AGCTACAGTG	TGCGCCAGGG	CTCAAGCTCC	ACCTCCATCG	5150
TGGGACCAAA	TGTGGAAGTG	TCTCATACGG	CTGAAACCTA	CACTGCACGG	5200
GCCAACACCC	CTGCTGTATA	GGCTAGGAGC	CGTCCAAAAT	GAGGTCATCC	5250
TCACACACCC	CATAACTAAA	TACATCATGG	CATGCATGTC	GGCTGACCTG	5300
GAGGTCGTCA	CTAGCACCTG	GGTGCTGGTA	GGCGGAGTCC	TTGCAGCTTT	5350
GGCCGCATAC	TGCCTGACGA	CAGGCAGTGT	GGTCATTGTG	GGCAGGATCA	5400
TCTTGTCCGG	GAAGCCAGCT	GTCGTTCCCG	ACAGGGAAGT	CCTCTACCAG	5450
GAGTTCGATG	AGATGGAAGA	GTGTGCCTCA	CAACTTCCTT	ACATCGAGCA	5500
GGGAATGCAG	CTCGCCGAGC	AATTCAAGCA	AAAGGCGCTC	GGGTTGTTGC	5550
AAACGGCCAC	CAAGCAAGCG	GAGGCTGCTG	CTCCCGTGGT	GGAGTCCAAG	5600
TGGCGAGCCC	TTGAGACCTT	CTGGGCGAAG	CACATGTGGA	ATTTCATCAG	5650
CGGAATACAG	TACCTAGCAG	GCTTATCCAC	TCTGCCTGGA	AACCCCGCGA	5700

FIG. 4C

HC-J4

10 1234567890	20 1234567890	30 1234567890	40 1234567890	50 1234567890	
TAGCATCATT	GATGGCATTT	ACAGCTTCTA	TCACTAGCCC	GCTCACCACC	5750
CAAAACACCC	TCCTGTTTAA	CATCTTGGGG	GGATGGGTGG	CTGCCCAACT	5800
CGCTCCTCCC	AGCGCTGCGT	CAGCTTTCGT	GGGCGCCGGC	ATCGCCGGAG	5850
CGGCTGTTGG	CAGCATAGGC	CTTGGGAAGG	TGCTCGTGGA	CATCTTGGCG	5900
GGCTATGGGG	CAGGGGTAGC	CGGCGCACTC	GTGGCCTTTA	AGGTCATGAG	5950
CGGCGAGGTG	CCCTCCACCG	AGGACCTGGT	CAACTTACTC	CCTGCCATCC	6000
TCTCTCCTGG	TGCCCTGGTC	GTCGGGGTCG	TGTGCGCAGC	AATACTGCGT	6050
CGGCACGTGG	GCCCGGGAGA	GGGGGCTGTG	CAGTGGATGA	ACCGGCTGAT	6100
AGCGTTCGCT	TCGCGGGGTA	ACCACGTCTC	CCCTACGCAC	TATGTGCCTG	6150
AGAGCGACGC	TGCAGCACGT	GTCACTCAGA	TCCTCTCTAG	CCTTACCATC	6200
ACTCAACTGC	TGAAGCGGCT	CCACCAGTGG	ATTAATGAGG	ACTGCTCTAC	6250
GCCATGCTCC	GGCTCGTGGC	TAAGGGATGT	TTGGGATTGG	ATATGCACGG	6300
TGTTGACTGA	CTTCAAGACC	TGGCTCCAGT	CCAAACTCCT	GCCGCGGTTA	6350
CCGGGAGTCC	CTTTCCTGTC	ATGCCAACGC	GGGTACAAGG	GAGTCTGGCG	6400
GGGGGACGGC	ATCATGCAAA	CCACCTGCCC	ATGCGGAGCA	CAGATCGCCG	6450
GACATGTCAA	AAACGGTTCC	ATGAGGATCG	TAGGGCCTAG	AACCTGCAGC	6500
AACACGTGGC	ACGGAACGTT	CCCCATCAAC	GCATACACCA	CGGGACCTTG	6550
CACACCCTCC	CCGGCGCCCA	ACTATTCCAG	GGCGCTATGG	CGGGTGGCTG	6600
CTGAGGAGTA	CGTGGAGGTT	ACGCGTGTGG	GGGATTTCCA	CTACGTGACG	6650
GGCATGACCA	CTGACAACGT	AAAGTGCCCA	TGCCAGGTTC	CGGCCCCCGA	6700
ATTCTTCACG	GAGGTGGATG	GAGTGCGGTT	GCACAGGTAC	GCTCCGGCGT	6750
GCAAACCTCT	TCTACGGGAG	GACGTCACGT	TCCAGGTCGG	GCTCAACCAA	6800
TACTTGGTCG	GGTCGCAGCT	CCCATGCGAG	CCCGAACCGG	ACGTAACAGT	6850
GCTTACTTCC	ATGCTCACCG	ATCCCTCCCA	CATTACAGCA	GAGACGGCTA	6900
AGCGTAGGCT	GGCTAGAGGG	TCTCCCCCCT	CTTTAGCCAG	CTCATCAGCT	6950
AGCCAGTTGT	CTGCGCCTTC	TTTGAAGGCG	ACATGCACTA	CCCACCATGA	7000
CTCCCCGGAC	GCTGACCTCA	TCGAGGCCAA	CCTCTTGTGG	CGGCAGGAGA	7050
TGGGCGGAAA	CATCACTCGC	GTGGAGTCAG	AGAATAAGGT	AGTAATTCTG	7100
GACTCTTTCG	AACCGCTTCA	CGCGGAGGGG	GATGAGAGGG	AGATATCCGT	7150
CGCGGCGGAG	ATCCTGCGAA	AATCCAGGAA	GTTCCCCTCA	GCGTTGCCCA	7200
TATGGGCACG	CCCGGACTAC	AATCCTCCAC	TGCTAGAGTC	CTGGAAGGAC	7250
CCGGACTACG	TCCCTCCGGT	GGTACACGGA	TGCCCATTGC	CACCTACCAA	7300
GGCTCCTCCA	ATACCACCTC	CACGGAGAAA	GAGGACGGTT	GTCCTGACAG	7350
AATCCAATGT	GTCTTCTGCC	TTGGCGGAGC	TCGCCACTAA	GACCTTCGGT	7400
AGCTCCGGAT	CGTCGGCCGT	TGATAGCGGC	ACGGCGACCG	CCCTTCCTGA	7450
CCTGGCCTCC	GACGACGGTG	ACAAAGGATC	CGACGTTGAG	TCGTACTCCT	7500
CCATGCCCCC	CCTTGAAGGG	GAGCCGGGGG	ACCCCGATCT	CAGCGACGGG	7550
TCTTGGTCTA	CCGTGAGTGA	GGAGGCTAGT	GAGGATGTCG	TCTGCTGCTC	7600

FIG. 4D

HC-J4

10 1234567890	20 1234567890	30 1234567890	40 1234567890	50 1234567890	
AATGTCCTAT	ACGTGGACAG	GCGCCCTGAT	CACGCCATGC	GCTGCGGAGG	7650
AAAGTAAGCT	GCCCATCAAC	CCGTTGAGCA	ACTCTTTGCT	GCGTCACCAC	7700
AACATGGTCT	ACGCCACAAC	ATCCCGCAGC	GCAAGCCTCC	GGCAGAAGAA	7750
GGTCACCTTT	GACAGATTGC	AAGTCCTGGA	TGATCATTAC	CGGGACGTAC	7800
TCAAGGAGAT	GAAGGCGAAG	GCGTCCACAG	TTAAGGCTAA	GCTTCTATCT	7850
ATAGAGGAGG	CCTGCAAGCT	GACGCCCCCA	CATTCGGCCA	AATCCAAATT	7900
TGGCTATGGG	GCAAAGGACG	TCCGGAACCT	ATCCAGCAGG	GCCGTTAACC	7950
ACATCCGCTC	CGTGTGGGAG	GACTTGCTGG	AAGACACTGA	AACACCAATT	8000
GACACCACCA	TCATGGCAAA	AAGTGAGGTT	TTCTGCGTCC	AACCAGAGAA	8050
GGGAGGCCGC	AAGCCAGCTC	GCCTTATCGT	ATTCCCAGAC	CTGGGAGTTC	8100
GTGTATGCGA	GAAGATGGCC	CTTTACGACG	TGGTCTCCAC	CCTTCCTCAG	8150
GCCGTGATGG	GCTCCTCATA	CGGATTTCAA	TACTCCCCCA	AGCAGCGGGT	8200
CGAGTTCCTG	GTGAATACCT	GGAAATCAAA	GAAATGCCCT	ATGGGCTTCT	8250
CATATGACAC	CCGCTGTTTT	GACTCAACGG	TCACTGAGAG	TGACATTCGT	8300
GTTGAGGAGT	CAATTTACCA	ATGTTGTGAC	TTGGCCCCCG	AGGCCAGACA	8350
GGCCATAAGG	TCGCTCACAG	AGCGGCTTTA	CATCGGGGGT	CCCCTGACTA	8400
ACTCAAAAGG	GCAGAACTGC	GGTTATCGCC	GGTGCCGCGC	AAGTGGCGTG	8450
CTGACGACTA	GCTGCGGTAA	TACCCTCACA	TGTTACTTGA	AGGCCACTGC	8500
AGCCTGTCGA	GCTGCAAAGC	TCCAGGACTG	CACGATGCTC	GTGAACGGAG	8550
ACGACCTTGT	CGTTATCTGT	GAAAGCGCGG	GAACCCAGGA	GGATGCGGCG	8600
GCCCTACGAG	CCTTCACGGA	GGCTATGACT	AGGTATTCCG	CCCCCCCCGG	8650
GGATCCGCCC	CAACCAGAAT	ACGACCTGGA	GCTGATAACA	TCATGTTCCT	8700
CCAATGTGTC	AGTCGCGCAC	GATGCATCTG	GCAAAAGGGT	ATACTACCTC	8750
ACCCGTGACC	CCACCACCCC	CCTTGCACGG	GCTGCGTGGG	AGACAGCTAG	8800
ACACACTCCA	ATCAACTCTT	GGCTAGGCAA	TATCATCATG	TATGCGCCCA	8850
CCCTATGGGC	AAGGATGATT	CTGATGACTC	ACTTTTTCTC	CATCCTTCTA	8900
GCTCAAGAGC	AACTTGAAAA	AGCCCTGGAT	TGTCAGATCT	ACGGGGCTTG	8950
CTACTCCATT	GAGCCACTTG	ACCTACCTCA	GATCATTGAA	CGACTCCATG	9000
GTCTTAGCGC	ATTTACACTC	CACAGTTACT	CTCCAGGTGA	GATCAATAGG	9050
GTGGCTTCAT	GCCTCAGGAA	ACTTGGGGTA	CCACCCTTGC	GAACCTGGAG	9100
ACATCGGGCC	AGAAGTGTCC	GCGCTAAGCT	ACTGTCCCAG	GGGGGGAGGG	9150
CCGCCACTTG	TGGCAGATAC	CTCTTTAACT	GGGCAGTAAG	GACCAAGCTT	9200
AAACTCACTC	CAATCCCGGC	CGCGTCCCAG	CTGGACTTGT	CTGGCTGGTT	9250
CGTCGCTGGT	TACAGCGGGG	GAGACATATA	TCACAGCCTG	TCTCGTGCCC	9300
GACCCCGCTG	GTTTCCGTTG	TGCCTACTCC	TACTTTCTGT	AGGGGTAGGC	9350
ATTTACCTGC	TCCCCAACCG	ATGAACGGGG	AGCTAACCAC	TCCAGGCCTT	9400
AAGCCATTTC	CTGTTTTTTT	TTTTTTTTTT	TTTTTTTTTT	TCTTTTTTTT	9450
TTTCTTTCCT	TTCCTTCTTT	TTTTCCTTTC	TTTTTCCCTT	CTTTAATGGT	9500

FIG. 4E

10	20	30	40	50	
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GGCTCCATCT	TAGCCCTAGT	CACGGCTAGC	TGTGAAAGGT	CCGTGAGCCG	9550
CATGACTGCA	GAGAGTGCTG	ATACTGGCCT	CTCTGCAGAT	CATGT	9595

FIG. 4F

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	KASERSQPRG	RRQPIPKARR	PEGRAWAQPG	YPWPLYGNEG	LGWAGWLLSP	100
	RGSRPSWGPT	DPRRRSRNLG	KVIDTLTCGF	ADLMGYIPLV	GAPLGGAARA	150
	LAHGVRVLED	GVNYATGNLP	GCSFSIFLLA	LLSCLTIPAS	AYEVRNVSGI	200
	YHVTNDCSNS	SIVYEAADVI	MHTPGCVPCV	QEGNSSRCWV	ALTPTLAARN	250
	ASVPTTTIRR	HVDLLVGTAA	FCSAMYVGDL	CGSIFLVSQL	FTFSPRRHET	300
	VQDCNCSIYP	GHVSGHRMAW	DMMMNWSPTT	ALVVSQLLRI	PQAVVDMVAG	350
	AHWGVLAGLA	YYSMVGNWAK	VLIVALLFAG	VDGETHTTGR	VAGHTTSGFT	400
	SLFSSGASQK	IQLVNTNGSW	HINRTALNCN	DSLQTGFFAA	LFYAHKFNSS	450
	GCPERMASCR	PIDWFAQGWG	PITYTKPNSS	DQRPYCWHYA	PRPCGVVPAS	500
	QVCGPVYCFT	PSPVVVGTTD	RSGVPTYSWG	ENETDVMLLN	NTRPPQGNWF	550
	GCTWMNSTGF	TKTCGGPPCN	IGGVGNRTLI	CPTDCFRKHP	EATYTKCGSG	600
	PWLTPRCLVD	YPYRLWHYPC	TLNFSIFKVR	MYVGGVEHRL	NAACNWTRGE	650
	RCNLEDRDRS	ELSPLLLSTT	EWQILPCAFT	TLPALSTGLI	HLHQNIVDVQ	700
	YLYGVGSAFV	SFAIKWEYIL	LLFLLLADAR	VCACLWMMLL	IAQAEAALEN	750
	LVVLNAASVA	GAHGILSFLV	FFCAAWYIKG	RLAPGAAYAF	YGVWPLLLLL	800
	LALPPRAYAL	DREMAASCGG	AVLVGLVFLT	LSPYYKVFLT	RLIWWLQYFI	850
	TRAEAHMQVW	VPPLNVRGGR	DAIILLTCAV	HPELIFDITK	LLLAILGPLM	900
	VLQAGITRVP	YFVRAQGLIR	ACMLVRKVAG	GHYVQMVFMK	LGALTGTYVY	950
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	PVSARRGKEI	FLGPADSLEG	QGWRLLAPIT	AYSQQTRGVL	GCIITSLTGR	1050
	DKNQVEGEVQ	VVSTATQSFL	ATCINGVCWT	VYHGAGSKTL	AGPKGPITQM	1100
	YTNVDLDLVG	WQAPPGARSM	TPCSCGSSDL	YLVTRHADVI	PVRRRGDSRG	1150
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	METTMRSPVF	TDNSTPPAVP	QTFQVAHLHA	PTGSGKSTKV	PAAYAAQGYK	1250
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	ADLEVVTSTW	VLVGGVLAAL	AAYCLTTGSV	VIVGRIILSG	KPAVVPDREV	1700
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	ESKWRALETF	WAKHMWNFIS	GIQYLAGLST	LPGNPAIASL	MAFTASITSP	1800
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FIG. 4G

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FIG. 4H

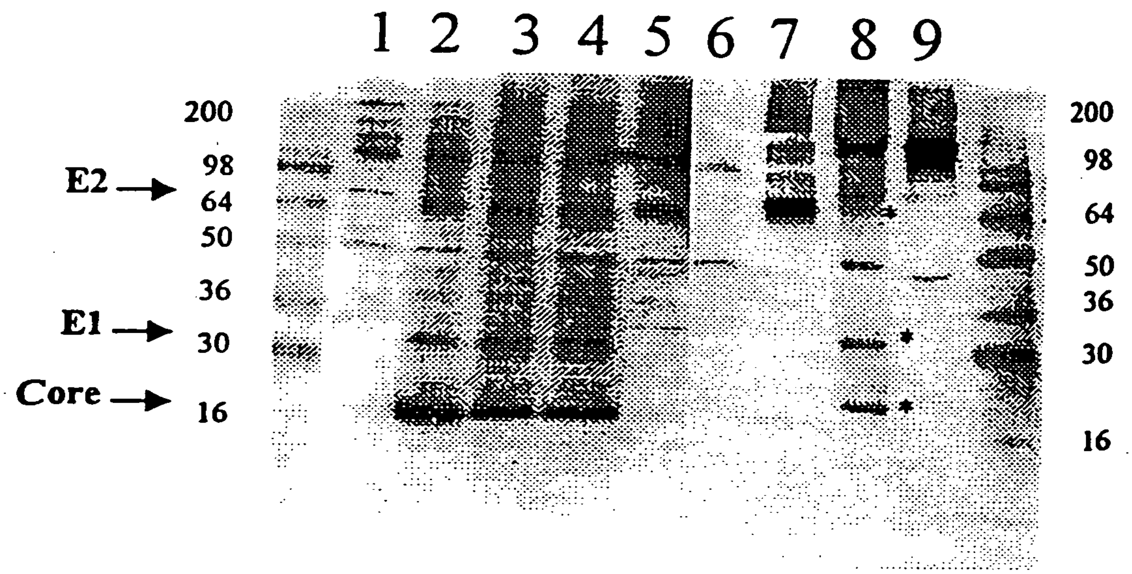

FIG. 5

SEQUENCE LISTING

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Bukh, Jens
Emerson, Suzanne
Purcell, Robert

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20 25 30

Phe Gly Glu Arg Gly Ala Val His Pro Gln Ser Thr Leu Lys Leu Pro
35 40 45

His Lys Arg Gly Glu Arg Asp Val Pro Thr Asn Leu Ala Ser Leu Pro
50 55 60

Lys Arg Gly Asp Cys Arg Ser Gly Asn Ser Arg Gly Pro Val Ser Gly
65 70 75 80

Ile Tyr Leu Lys Pro Gly Pro Leu Phe Tyr Gln Asp Tyr Lys Gly Pro
85 90 95

Val Tyr His Arg Ala Pro Leu Glu Leu Phe Glu Glu Gly Ser Met Cys
100 105 110

Glu Thr Thr Lys Arg Ile Gly Arg Val Thr Gly Ser Asp Gly Lys Leu
115 120 125

Tyr His Ile Tyr Val Cys Ile Asp Gly Cys Ile Ile Ile Lys Ser Ala
130 135 140

Thr Arg Ser Tyr Gln Arg Val Phe Arg Trp Val His Asn Arg Leu Asp
145 150 155 160

Cys Pro Leu Trp Val Thr Ser Cys Ser Thr Asn Pro Lys Pro Gln Arg
165 170 175

Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln Asp Val Lys Phe Pro
180 185 190

Gly Gly Gly Gln Ile Val Gly Gly Val Tyr Leu Leu Pro Arg Arg Gly
195 200 205

Pro Arg Leu Gly Val Arg Ala Thr Arg Lys Thr Ser Glu Arg Ser Gln
210 215 220

Pro Arg Gly Arg Arg Gln Pro Ile Pro Lys Ala Arg Arg Pro Glu Gly
225 230 235 240

Arg Thr Trp Ala Gln Pro Gly Tyr Pro Trp Pro Leu Tyr Gly Asn Glu
245 250 255

Gly Cys Gly Trp Ala Gly Trp Leu Leu Ser Pro Arg Gly Ser Arg Pro
260 265 270

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Ser Trp Gly Pro Thr Asp Pro Arg Arg Arg Ser Arg Asn Leu Gly Lys
        275                 280                     285

Val Ile Asp Thr Leu Thr Cys Gly Phe Ala Asp Leu Met Gly Tyr Ile
    290                 295                     300

Pro Leu Val Gly Ala Pro Leu Gly Gly Ala Ala Arg Ala Leu Ala His
305                     310                     315                 320

Gly Val Arg Val Leu Glu Asp Gly Val Asn Tyr Ala Thr Gly Asn Leu
                325                 330                     335

Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu Ala Leu Leu Ser Cys Leu
            340                 345                     350

Thr Val Pro Ala Ser Ala Tyr Gln Val Arg Asn Ser Ser Gly Leu Tyr
        355                 360                     365

His Val Thr Asn Asp Cys Pro Asn Ser Ser Ile Val Tyr Glu Ala Ala
    370                 375                     380

Asp Ala Ile Leu His Thr Pro Gly Cys Val Pro Cys Val Arg Glu Gly
385                     390                     395                 400

Asn Ala Ser Arg Cys Trp Val Ala Val Thr Pro Thr Val Ala Thr Arg
                405                 410                     415

Asp Gly Lys Leu Pro Thr Thr Gln Leu Arg Arg His Ile Asp Leu Leu
            420                 425                     430

Val Gly Ser Ala Thr Leu Cys Ser Ala Leu Tyr Val Gly Asp Leu Cys
        435                 440                     445

Gly Ser Val Phe Leu Val Gly Gln Leu Phe Thr Phe Ser Pro Arg Arg
    450                 455                     460

His Trp Thr Thr Gln Asp Cys Asn Cys Ser Ile Tyr Pro Gly His Ile
465                     470                     475                 480

Thr Gly His Arg Met Ala Trp Asp Met Met Met Asn Trp Ser Pro Thr
                485                 490                     495

Ala Ala Leu Val Val Ala Gln Leu Leu Arg Ile Pro Gln Ala Ile Met
            500                 505                     510

Asp Met Ile Ala Gly Ala His Trp Gly Val Leu Ala Gly Ile Ala Tyr
        515                 520                     525
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Phe Ser Met Val Gly Asn Trp Ala Lys Val Leu Val Val Leu Leu Leu
    530                 535                 540

Phe Ala Gly Val Asp Ala Glu Thr His Val Thr Gly Gly Asn Ala Gly
545                 550                 555                 560

Arg Thr Thr Ala Gly Leu Val Gly Leu Leu Thr Pro Gly Ala Lys Gln
                565                 570                 575

Asn Ile Gln Leu Ile Asn Thr Asn Gly Ser Trp His Ile Asn Ser Thr
            580                 585                 590

Ala Leu Asn Cys Asn Glu Ser Leu Asn Thr Gly Trp Leu Ala Gly Leu
        595                 600                 605

Phe Tyr Gln His Lys Phe Asn Ser Ser Gly Cys Pro Glu Arg Leu Ala
    610                 615                 620

Ser Cys Arg Arg Leu Thr Asp Phe Ala Gln Gly Trp Gly Pro Ile Ser
625                 630                 635                 640

Tyr Ala Asn Gly Ser Gly Leu Asp Glu Arg Pro Tyr Cys Trp His Tyr
                645                 650                 655

Pro Pro Arg Pro Cys Gly Ile Val Pro Ala Lys Ser Val Cys Gly Pro
            660                 665                 670

Val Tyr Cys Phe Thr Pro Ser Pro Val Val Val Gly Thr Thr Asp Arg
        675                 680                 685

Ser Gly Ala Pro Thr Tyr Ser Trp Gly Ala Asn Asp Thr Asp Val Phe
    690                 695                 700

Val Leu Asn Asn Thr Arg Pro Pro Leu Gly Asn Trp Phe Gly Cys Thr
705                 710                 715                 720

Trp Met Asn Ser Thr Gly Phe Thr Lys Val Cys Gly Ala Pro Pro Cys
                725                 730                 735

Val Ile Gly Gly Val Gly Asn Asn Thr Leu Leu Cys Pro Thr Asp Cys
            740                 745                 750

Phe Arg Lys His Pro Glu Ala Thr Tyr Ser Arg Cys Gly Ser Gly Pro
        755                 760                 765

Trp Ile Thr Pro Arg Cys Met Val Asp Tyr Pro Tyr Arg Leu Trp His
    770                 775                 780
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```
Tyr Pro Cys Thr Ile Asn Tyr Thr Ile Phe Lys Val Arg Met Tyr Val
785                 790                 795                 800

Gly Gly Val Glu His Arg L u Glu Ala Ala Cys Asn Trp Thr Arg Gly
                805                 810                 815

Glu Arg Cys Asp Leu Glu Asp Arg Asp Arg Ser Glu Leu Ser Pro Leu
            820                 825                 830

Leu Leu Ser Thr Thr Gln Trp Gln Val Leu Pro Cys Ser Phe Thr Thr
        835                 840                 845

Leu Pro Ala Leu Ser Thr Gly Leu Ile His Leu His Gln Asn Ile Val
    850                 855                 860

Asp Val Gln Tyr Leu Tyr Gly Val Gly Ser Ser Ile Ala Ser Trp Ala
865                 870                 875                 880

Ile Lys Trp Glu Tyr Val Val Leu Leu Phe Leu Leu Leu Ala Asp Ala
                885                 890                 895

Arg Val Cys Ser Cys Leu Trp Met Met Leu Leu Ile Ser Gln Ala Glu
            900                 905                 910

Ala Ile Gln Tyr Gly Ser Gly Glu Val Val Met Met Gly Asn Leu Leu
        915                 920                 925

Thr His Asn Asn Ile Glu Val Val Thr Tyr Phe Leu Leu Leu Tyr Leu
    930                 935                 940

Leu Leu Arg Glu Glu Ser Val Lys Lys Trp Val Leu Leu Leu Tyr His
945                 950                 955                 960

Ile Leu Val Val His Pro Ile Lys Ser Val Ile Val Ile Leu Leu Met
                965                 970                 975

Ile Gly Asp Val Val Lys Ala Asp Ser Gly Gly Gln Glu Tyr Leu Gly
            980                 985                 990

Lys Ile Asp Leu Cys Phe Thr Thr Val Val Leu Ile Val Ile Gly Leu
        995                1000                1005

Ile Ile Ala Arg Arg Asp Pro Thr Ile Val Pro Leu Val Thr Ile Met
   1010                1015                1020

Ala Ala Leu Arg Val Thr Glu Leu Thr His Gln Pro Gly Val Asp Ile
1025               1030                1035                1040
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Ala Val Ala Val Met Thr Ile Thr Leu Leu Met Val Ser Tyr Val Thr
1045 1050 1055

Asp Tyr Phe Arg Tyr Lys Lys Trp Leu Gln Cys Ile Leu Ser Leu Val
1060 1065 1070

Ser Gly Val Phe Leu Ile Arg Ser Leu Ile Tyr Leu Gly Arg Ile Glu
1075 1080 1085

Met Pro Glu Val Thr Ile Pro Asn Trp Arg Pro Leu Thr Leu Ile Leu
1090 1095 1100

Leu Tyr Leu Ile Ser Thr Thr Ile Val Thr Arg Trp Lys Val Asp Val
1105 1110 1115 1120

Ala Gly Leu Leu Leu Gln Cys Val Pro Ile Leu Leu Leu Val Thr Thr
1125 1130 1135

Leu Trp Ala Asp Phe Leu Thr Leu Ile Leu Ile Leu Pro Thr Tyr Glu
1140 1145 1150

Leu Val Lys Leu Tyr Tyr Leu Lys Thr Val Arg Thr Asp Ile Glu Arg
1155 1160 1165

Ser Trp Leu Gly Gly Ile Asp Tyr Thr Arg Val Asp Ser Ile Tyr Asp
1170 1175 1180

Val Asp Glu Ser Gly Glu Gly Val Tyr Leu Phe Pro Ser Arg Gln Lys
1185 1190 1195 1200

Ala Gln Gly Asn Phe Ser Ile Leu Leu Pro Leu Ile Lys Ala Thr Leu
1205 1210 1215

Ile Ser Cys Val Ser Ser Lys Trp Gln Leu Ile Tyr Met Ser Tyr Leu
1220 1225 1230

Thr Leu Asp Phe Met Tyr Tyr Met His Arg Lys Val Ile Glu Glu Ile
1235 1240 1245

Ser Gly Gly Thr Asn Ile Ile Ser Arg Leu Val Ala Ala Leu Ile Glu
1250 1255 1260

Leu Asn Trp Ser Met Glu Glu Glu Glu Ser Lys Gly Leu Lys Lys Phe
1265 1270 1275 1280

Tyr Leu Leu Ser Gly Arg Leu Arg Asn Leu Ile Ile Lys His Lys Val
1285 1290 1295

Arg Asn Glu Thr Val Ala Ser Trp Tyr Gly Glu Glu Glu Val Tyr Gly
1300 1305 1310

Met Pro Lys Ile Met Thr Ile Ile Lys Ala Ser Thr Leu Ser Lys Ser
1315 1320 1325

Arg His Cys Ile Ile Cys Thr Val Cys Glu Gly Arg Glu Trp Lys Gly
1330 1335 1340

Gly Thr Cys Pro Lys Cys Gly Arg His Gly Lys Pro Ile Thr Cys Gly
1345 1350 1355 1360

Met Ser Leu Ala Asp Phe Glu Glu Arg His Tyr Lys Arg Ile Phe Ile
1365 1370 1375

Arg Glu Gly Asn Phe Glu Gly Met Cys Ser Arg Cys Gln Gly Lys His
1380 1385 1390

Arg Arg Phe Glu Met Asp Arg Glu Pro Lys Ser Ala Arg Tyr Cys Ala
1395 1400 1405

Glu Cys Asn Arg Leu His Pro Ala Glu Glu Gly Asp Phe Trp Ala Glu
1410 1415 1420

Ser Ser Met Leu Gly Leu Lys Ile Thr Tyr Phe Ala Leu Met Asp Gly
1425 1430 1435 1440

Lys Val Tyr Asp Ile Thr Glu Trp Ala Gly Cys Gln Arg Val Gly Ile
1445 1450 1455

Ser Pro Asp Thr His Arg Val Pro Cys His Ile Ser Phe Gly Ser Arg
1460 1465 1470

Met Pro Phe Arg Gln Glu Tyr Asn Gly Phe Val Gln Tyr Thr Ala Arg
1475 1480 1485

Gly Gln Leu Phe Leu Arg Asn Leu Pro Val Leu Ala Thr Lys Val Lys
1490 1495 1500

Met Leu Met Val Gly Asn Leu Gly Glu Glu Ile Gly Asn Leu Glu His
1505 1510 1515 1520

Leu Gly Trp Ile Leu Arg Gly Pro Ala Val Cys Lys Lys Ile Thr Glu
1525 1530 1535

His Glu Lys Cys His Ile Asn Ile Leu Asp Lys Leu Thr Ala Phe Phe
1540 1545 1550

Gly Ile Met Pro Arg Gly Thr Thr Pro Arg Ala Pro Val Arg Phe Pro
1555 1560 1565

Thr Ser Leu Leu Lys Val Arg Arg Gly Leu Glu Thr Gly Trp Ala Tyr
1570 1575 1580

Thr His Gln Gly Gly Ile Ser Ser Val Asp His Val Thr Ala Gly Lys
1585 1590 1595 1600

Asp Leu Leu Val Cys Asp Ser Met Gly Arg Thr Arg Val Val Cys Gln
1605 1610 1615

Ser Asn Asn Arg Leu Thr Asp Glu Thr Glu Tyr Gly Val Lys Thr Asp
1620 1625 1630

Ser Gly Cys Pro Asp Gly Ala Arg Cys Tyr Val Leu Asn Pro Glu Ala
1635 1640 1645

Val Asn Ile Ser Gly Ser Lys Gly Ala Val Val His Leu Gln Lys Thr
1650 1655 1660

Gly Gly Glu Phe Thr Cys Val Thr Ala Ser Gly Thr Pro Ala Phe Phe
1665 1670 1675 1680

Asp Leu Lys Asn Leu Lys Gly Trp Ser Gly Leu Pro Ile Phe Glu Ala
1685 1690 1695

Ser Ser Gly Arg Val Val Gly Arg Val Lys Val Gly Lys Asn Glu Glu
1700 1705 1710

Ser Lys Pro Thr Lys Ile Met Ser Gly Ile Gln Thr Val Ser Lys Asn
1715 1720 1725

Thr Ala Asp Leu Thr Glu Met Val Lys Lys Ile Thr Ser Met Asn Arg
1730 1735 1740

Gly Asp Phe Lys Gln Ile Thr Leu Ala Thr Gly Ala Gly Lys Thr Thr
1745 1750 1755 1760

Glu Leu Pro Lys Ala Val Ile Glu Glu Ile Gly Arg His Lys Arg Val
1765 1770 1775

Leu Val Leu Ile Pro Leu Arg Ala Ala Ala Glu Ser Val Tyr Gln Tyr
1780 1785 1790

Met Arg Leu Lys His Pro Ser Ile Ser Phe Asn Leu Arg Ile Gly Asp
1795 1800 1805

Met Lys Glu Gly Asp Met Ala Thr Gly Ile Thr Tyr Ala Ser Tyr Gly
1810 1815 1820

Tyr Phe Cys Gln Met Pro Gln Pro Lys Leu Arg Ala Ala Met Val Glu
1825 1830 1835 1840

Tyr Ser Tyr Ile Phe Leu Asp Glu Tyr His Cys Ala Thr Pro Glu Gln
1845 1850 1855

Leu Ala Ile Ile Gly Lys Ile His Arg Phe Ser Glu Ser Ile Arg Val
1860 1865 1870

Val Ala Met Thr Ala Thr Pro Ala Gly Ser Val Thr Thr Thr Gly Gln
1875 1880 1885

Lys His Pro Ile Glu Glu Phe Ile Ala Pro Glu Val Met Lys Gly Glu
1890 1895 1900

Asp Leu Gly Ser Gln Phe Leu Asp Ile Ala Gly Leu Lys Ile Pro Val
1905 1910 1915 1920

Asp Glu Met Lys Gly Asn Met Leu Val Phe Val Pro Thr Arg Asn Met
1925 1930 1935

Ala Val Glu Val Ala Lys Lys Leu Lys Ala Lys Gly Tyr Asn Ser Gly
1940 1945 1950

Tyr Tyr Tyr Ser Gly Glu Asp Pro Ala Asn Leu Arg Val Val Thr Ser
1955 1960 1965

Gln Ser Pro Tyr Val Ile Val Ala Thr Asn Ala Ile Glu Ser Gly Val
1970 1975 1980

Thr Leu Pro Asp Leu Asp Thr Val Ile Asp Thr Gly Leu Lys Cys Glu
1985 1990 1995 2000

Lys Arg Val Arg Val Ser Ser Lys Ile Pro Phe Ile Val Thr Gly Leu
2005 2010 2015

Lys Arg Met Ala Val Thr Val Gly Glu Gln Ala Gln Arg Arg Gly Arg
2020 2025 2030

Val Gly Arg Val Lys Pro Gly Arg Tyr Tyr Arg Ser Gln Glu Thr Ala
2035 2040 2045

Thr Gly Ser Lys Asp Tyr His Tyr Asp Leu Leu Gln Ala Gln Arg Tyr
2050 2055 2060

Gly Ile Glu Asp Gly Ile Asn Val Thr Lys Ser Phe Arg Glu Met Asn
2065 2070 2075 2080

Tyr Asp Trp Ser Leu Tyr Glu Glu Asp Ser Leu Leu Ile Thr Gln Leu
2085 2090 2095

Glu Ile Leu Asn Asn Leu Leu Ile Ser Glu Asp Leu Pro Ala Ala Val
2100 2105 2110

Lys Asn Ile Met Ala Arg Thr Asp His Pro Glu Pro Ile Gln Leu Ala
2115 2120 2125

Tyr Asn Ser Tyr Glu Val Gln Val Pro Val Leu Phe Pro Lys Ile Arg
2130 2135 2140

Asn Gly Glu Val Thr Asp Thr Tyr Glu Asn Tyr Ser Phe Leu Asn Ala
2145 2150 2155 2160

Arg Lys Leu Gly Glu Asp Val Pro Val Tyr Ile Tyr Ala Thr Glu Asp
2165 2170 2175

Glu Asp Leu Ala Val Asp Leu Leu Gly Leu Asp Trp Pro Asp Pro Gly
2180 2185 2190

Asn Gln Gln Val Val Glu Thr Gly Lys Ala Leu Lys Gln Val Thr Gly
2195 2200 2205

Leu Ser Ser Ala Glu Asn Ala Leu Leu Val Ala Leu Phe Gly Tyr Val
2210 2215 2220

Gly Tyr Gln Ala Leu Ser Lys Arg His Val Pro Met Ile Thr Asp Ile
2225 2230 2235 2240

Tyr Thr Ile Glu Asp Gln Arg Leu Glu Asp Thr Thr His Leu Gln Tyr
2245 2250 2255

Ala Pro Asn Ala Ile Lys Thr Asp Gly Thr Glu Thr Glu Leu Lys Glu
2260 2265 2270

Leu Ala Ser Gly Asp Val Glu Lys Ile Met Gly Ala Ile Ser Asp Tyr
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Ala Ala Gly Gly Leu Glu Phe Val Lys Ser Gln Ala Glu Lys Ile Lys
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Thr Ala Pro Leu Phe Lys Glu Asn Ala Glu Ala Ala Lys Gly Tyr Val
2305 2310 2315 2320

Gln Lys Phe Ile Asp Ser Leu Ile Glu Asn Lys Glu Glu Ile Ile Arg
2325 2330 2335

Tyr Gly Leu Trp Gly Thr His Thr Ala Leu Tyr Lys Ser Ile Ala Ala
2340 2345 2350

Arg Leu Gly His Glu Thr Ala Phe Ala Thr Leu Val Leu Lys Trp Leu
2355 2360 2365

Ala Phe Gly Gly Glu Ser Val Ser Asp His Val Lys Gln Ala Ala Val
2370 2375 2380

Asp Leu Val Val Tyr Tyr Val Met Asn Lys Pro Ser Phe Pro Gly Asp
2385 2390 2395 2400

Ser Glu Thr Gln Gln Glu Gly Arg Arg Phe Val Ala Ser Leu Phe Ile
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Ser Ala Leu Ala Thr Tyr Thr Tyr Lys Thr Trp Asn Tyr His Asn Leu
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Ser Lys Val Val Glu Pro Ala Leu Ala Tyr Leu Pro Tyr Ala Thr Ser
2435 2440 2445

Ala Leu Lys Met Phe Thr Pro Thr Arg Leu Glu Ser Val Val Ile Leu
2450 2455 2460

Ser Thr Thr Ile Tyr Lys Thr Tyr Leu Ser Ile Arg Lys Gly Lys Ser
2465 2470 2475 2480

Asp Gly Leu Leu Gly Thr Gly Ile Ser Ala Ala Met Glu Ile Leu Ser
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Gln Asn Pro Val Ser Val Gly Ile Ser Val Met Leu Gly Val Gly Ala
2500 2505 2510

Ile Ala Ala His Asn Ala Ile Glu Ser Ser Glu Gln Lys Arg Thr Leu
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Leu Met Lys Val Phe Val Lys Asn Phe Leu Asp Gln Ala Ala Thr Asp
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Glu Leu Val Lys Glu Asn Pro Glu Lys Ile Ile Met Ala Leu Phe Glu
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Ala Val Gln Thr Ile Gly Asn Pro Leu Arg Leu Ile Tyr His Leu Tyr
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Gly Val Tyr Tyr Lys Gly Trp Glu Ala Lys Glu Leu Ser Glu Arg Thr
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Ala Gly Arg Asn Leu Phe Thr Leu Ile Met Phe Glu Ala Phe Glu Leu
2595 2600 2605

Leu Gly Met Asp Ser Gln Gly Lys Ile Arg Asn Leu Ser Gly Asn Tyr
2610 2615 2620

Ile Leu Asp Leu Ile Tyr Gly Leu His Lys Gln Ile Asn Arg Gly Leu
2625 2630 2635 2640

Lys Lys Met Val Leu Gly Trp Ala Pro Ala Pro Phe Ser Cys Asp Trp
2645 2650 2655

Thr Pro Ser Asp Glu Arg Ile Arg Leu Pro Thr Asp Asn Tyr Leu Arg
2660 2665 2670

Val Glu Thr Arg Cys Pro Cys Gly Tyr Glu Met Lys Ala Phe Lys Asn
2675 2680 2685

Val Gly Gly Lys Leu Thr Lys Val Glu Glu Ser Gly Pro Phe Leu Cys
2690 2695 2700

Arg Asn Arg Pro Gly Arg Gly Pro Val Asn Tyr Arg Val Thr Lys Tyr
2705 2710 2715 2720

Tyr Asp Asp Asn Leu Arg Glu Ile Lys Pro Val Ala Lys Leu Glu Gly
2725 2730 2735

Gln Val Glu His Tyr Tyr Lys Gly Val Thr Ala Lys Ile Asp Tyr Ser
2740 2745 2750

Lys Gly Lys Met Leu Leu Ala Thr Asp Lys Trp Glu Val Glu His Gly
2755 2760 2765

Val Ile Thr Arg Leu Ala Lys Arg Tyr Thr Gly Val Gly Phe Asn Gly
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Ala Tyr Leu Gly Asp Glu Pro Asn His Arg Ala Leu Val Glu Arg Asp
2785 2790 2795 2800

Cys Ala Thr Ile Thr Lys Asn Thr Val Gln Phe Leu Lys Met Lys Lys
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Gly Cys Ala Phe Thr Tyr Asp Leu Thr Ile Ser Asn Leu Thr Arg Leu
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Ile Glu Leu Val His Arg Asn Asn Leu Glu Glu Lys Glu Ile Pro Thr
2835 2840 2845

Ala Thr Val Thr Thr Trp Leu Ala Tyr Thr Phe Val Asn Glu Asp Val
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Gly Thr Ile Lys Pro Val Leu Gly Glu Arg Val Ile Pro Asp Pro Val
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Val Asp Ile Asn Leu Gln Pro Glu Val Gln Val Asp Thr Ser Glu Val
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Gly Ile Thr Ile Ile Gly Arg Glu Thr Leu Met Thr Thr Gly Val Thr
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Pro Val Leu Glu Lys Val Glu Pro Asp Ala Ser Asp Asn Gln Asn Ser
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Val Lys Ile Gly Leu Asp Glu Gly Asn Tyr Pro Gly Pro Gly Ile Gln
2930 2935 2940

Thr His Thr Leu Thr Glu Glu Ile His Asn Arg Asp Ala Arg Pro Phe
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Ile Met Ile Leu Gly Ser Arg Asn Ser Ile Ser Asn Arg Ala Lys Thr
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Ala Arg Asn Ile Asn Leu Tyr Thr Gly Asn Asp Pro Arg Glu Ile Arg
2980 2985 2990

Asp Leu Met Ala Ala Gly Arg Met Leu Val Val Ala Leu Arg Asp Val
2995 3000 3005

Asp Pro Glu Leu Ser Glu Met Val Asp Phe Lys Gly Thr Phe Leu Asp
3010 3015 3020

Arg Glu Ala Leu Glu Ala Leu Ser Leu Gly Gln Pro Lys Pro Lys Gln
3025 3030 3035 3040

Val Thr Lys Glu Ala Val Arg Asn Leu Ile Glu Gln Lys Lys Asp Val
3045 3050 3055

Glu Ile Pro Asn Trp Phe Ala Ser Asp Asp Pro Val Phe Leu Glu Val
3060 3065 3070

Ala Leu Lys Asn Asp Lys Tyr Tyr Leu Val Gly Asp Val Gly Glu Val
3075 3080 3085

Lys Asp Gln Ala Lys Ala Leu Gly Ala Thr Asp Gln Thr Arg Ile Ile
3090 3095 3100

Lys Glu Val Gly Ser Arg Thr Tyr Ala Met Lys Leu Ser Ser Trp Phe
3105 3110 3115 3120

Leu Gln Ala Ser Asn Lys Gln Met Ser Leu Thr Pro Leu Phe Glu Glu
3125 3130 3135

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Tyr Thr Trp Gly Glu Asn Glu Thr Asp Val Phe Leu Leu Asn Ser Thr
530 535 540

Arg Pro Pro Leu Gly Ser Trp Phe Gly Cys Thr Trp Met Asn Ser Ser
545 550 555 560

Gly Tyr Thr Lys Thr Cys Gly Ala Pro Pro Cys Arg Thr Arg Ala Asp
565 570 575

Phe Asn Ala Ser Thr Asp Leu Leu Cys Pro Thr Asp Cys Phe Arg Lys
580 585 590

His Pro Asp Thr Thr Tyr Leu Lys Cys Gly Ser Gly Pro Trp Leu Thr
595 600 605

Pro Arg Cys Leu Ile Asp Tyr Pro Tyr Arg Leu Trp His Tyr Pro Cys
610 615 620

Thr Val Asn Tyr Thr Ile Phe Lys Ile Arg Met Tyr Val Gly Gly Val
625 630 635 640

Glu His Arg Leu Thr Ala Ala Cys Asn Phe Thr Arg Gly Asp Arg Cys
645 650 655

Asn Leu Glu Asp Arg Asp Arg Ser Gln Leu Ser Pro Leu Leu His Ser
660 665 670

Thr Thr Glu Trp Ala Ile Leu Pro Cys Ser Tyr Ser Asp Leu Pro Ala
675 680 685

Leu Ser Thr Gly Leu Leu His Leu His Gln Asn Ile Val Asp Val Gln
690 695 700

Phe Met Tyr Gly Leu Ser Pro Ala Leu Thr Lys Tyr Ile Val Arg Trp
705 710 715 720

Glu Trp Val Ile Leu Leu Phe Leu Leu Leu Ala Asp Ala Arg Val Cys
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Ala Cys Leu Trp Met Leu Ile Leu Leu Gly Gln Ala Glu Ala Ala Leu
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Phe Leu Tyr Phe Val Ile Phe Phe Val Ala Ala Trp Tyr Ile Lys Gly
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Arg Val Val Pro Leu Ala Thr Tyr Ser Leu Thr Gly Leu Trp Ser Phe
785 790 795 800

Ser Leu Leu Leu Leu Ala Leu Pro Gln Gln Ala Tyr Ala Tyr Asp Ala
805 810 815

Ser Val His Gly Gln Ile Gly Ala Ala Leu Leu Val Met Ile Thr Leu
820 825 830

Phe Thr Leu Thr Pro Gly Tyr Lys Thr Leu Leu Ser Arg Phe Leu Trp
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Trp Leu Cys Tyr Leu Leu Thr Leu Gly Glu Ala Met Val Gln Glu Trp
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Val Ala Ile Phe Tyr Pro Gly Val Val Phe Asp Ile Thr Lys Trp Leu
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Val Pro Tyr Phe Val Arg Ala His Ala Leu Leu Arg Met Cys Thr Met
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Leu Gly Arg Trp Thr Gly Thr Tyr Ile Tyr Asp His Leu Thr Pro Met
945 950 955 960

Ser Asp Trp Ala Ala Ser Gly Leu Arg Asp Leu Ala Val Ala Val Glu
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Pro Ile Ile Phe Ser Pro Met Glu Lys Lys Val Ile Val Trp Gly Ala
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Glu Thr Ala Ala Cys Gly Asp Ile Leu His Gly Leu Pro Val Ser Ala
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Arg Leu Gly Arg Glu Val Leu Leu Gly Pro Ala Asp Gly Tyr Thr Ser
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Lys Gly Trp Ser Leu Leu Ala Pro Ile Thr Ala Tyr Ala Gln Gln Thr
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Thr Lys Ser Leu Glu Pro Cys Thr Cys Gly Ala Val Asp Leu Tyr Leu
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Arg Gly Ala Leu Leu Ser Pro Arg Pro Leu Ser Thr Leu Lys Gly Ser
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Ser Gly Gly Pro Val Leu Cys Pro Arg Gly His Ala Val Gly Val Phe
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Arg Ala Ala Val Cys Ser Arg Gly Val Ala Lys Ser Ile Asp Phe Ile
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Asn Ser Thr Pro Pro Ala Val Pro Gln Thr Tyr Gln Val Gly Tyr Leu
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Ala Ala Gln Gly Tyr Lys Val Leu Val Leu Asn Pro Ser Val Ala Ala
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Thr Leu Gly Phe Gly Ala Tyr Leu Ser Lys Ala His Gly Ile Asn Pro
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1285 1290 1295

Tyr Ser Thr Tyr Gly Lys Phe Leu Ala Asp Gly Gly Cys Ala Gly Gly
1300 1305 1310

Ala Tyr Asp Ile Ile Ile Cys Asp Glu Cys His Ala Val Asp Ser Thr
1315 1320 1325

Thr Ile Leu Gly Ile Gly Thr Val Leu Asp Gln Ala Glu Thr Ala Gly
1330 1335 1340

Val Arg Leu Thr Val Leu Ala Thr Ala Thr Pro Pro Gly Ser Val Thr
1345 1350 1355 1360

Thr Pro His Pro Asn Ile Glu Glu Val Ala Leu Gly Gln Glu Gly Glu
1365 1370 1375

Ile Pro Phe Tyr Gly Arg Ala Ile Pro Leu Ser Tyr Ile Lys Gly Gly
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Arg His Leu Ile Phe Cys His Ser Lys Lys Lys Cys Asp Glu Leu Ala
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Ala Ala Leu Arg Gly Met Gly Leu Asn Ser Val Ala Tyr Tyr Arg Gly
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Leu Asp Val Ser Val Ile Pro Thr Gln Gly Asp Val Val Val Val Ala
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Thr Asp Ala Leu Met Thr Gly Tyr Thr Gly Asp Phe Asp Ser Val Ile
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Asp Cys Asn Val Ala Val Thr Gln Val Val Asp Phe Ser Leu Asp Pro
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Thr Phe Thr Ile Thr Thr Gln Ile Val Pro Gln Asp Ala Val Ser Arg
1475 1480 1485

Ser Gln Arg Arg Gly Arg Thr Gly Arg Gly Arg Leu Gly Ile Tyr Arg
1490 1495 1500

Tyr Val Ser Thr Gly Glu Arg Ala Ser Gly Met Phe Asp Ser Val Val
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Leu Cys Glu Cys Tyr Asp Ala Gly Ala Ala Trp Tyr Glu Leu Thr Pro
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Ser Glu Thr Thr Val Arg Leu Arg Ala Tyr Phe Asn Thr Pro Gly Leu
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Pro Val Cys Gln Asp His Leu Glu Phe Trp Glu Ala Val Phe Thr Gly
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Arg Leu Lys Pro Thr Leu Val Gly Pro Thr Pro Leu Leu Tyr Arg Leu
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Gly Ser Val Thr Asn Glu Val Thr Leu Thr His Pro Val Thr Lys Tyr
1635 1640 1645

Ile Ala Thr Cys Met Gln Ala Asp Leu Glu Val Met Thr Ser Thr Trp
1650 1655 1660

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Glu Glu Cys Ala Ser Arg Ala Ala Leu Ile Glu Glu Gly Gln Arg Ile
1715 1720 1725

Ala Glu Met Leu Lys Ser Lys Ile Gln Gly Leu Leu Gln Gln Ala Ser
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1765 1770 1775

Gln Tyr Leu Ala Gly Leu Ser Thr Leu Pro Gly Asn Pro Ala Val Ala
1780 1785 1790

Ser Met Met Ala Phe Ser Ala Ala Leu Thr Ser Pro Leu Ser Thr Ser
1795 1800 1805

Thr Thr Ile Leu Leu Asn Ile Leu Gly Gly Trp Leu Ala Ser Gln Ile
1810 1815 1820

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1825 1830 1835 1840

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1845 1850 1855

Ala Gly Tyr Gly Ala Gly Ile Ser Gly Ala Leu Val Ala Phe Lys Ile
1860 1865 1870

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1875 1880 1885

Gly Ile Leu Ser Pro Gly Ala Leu Val Val Gly Val Ile Cys Ala Ala
1890 1895 1900

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1905 1910 1915 1920

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1925 1930 1935

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1940 1945 1950

Gly Ser Leu Thr Ile Thr Ser Leu Leu Arg Arg Leu His Asn Trp Ile
1955 1960 1965

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Arg Asp Ile Arg Thr Glu Glu Ser Ile Tyr Arg Ala Cys Ser Leu Pro
2675 2680 2685

Glu Glu Ala His Thr Ala Ile His Ser Leu Thr Glu Arg Leu Tyr Val
2690 2695 2700

Gly Gly Pro Met Phe Asn Ser Lys Gly Gln Thr Cys Gly Tyr Arg Arg
2705 2710 2715 2720

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Pro Thr Met Leu Val Cys Gly Asp Asp Leu Val Val Ile Ser Glu Ser
2755 2760 2765

Gln Gly Thr Glu Glu Asp Glu Arg Asn Leu Arg Ala Phe Thr Glu Ala
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2785 2790 2795 2800

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2945 2950 2955 2960

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Ala Arg Pro Arg Leu Leu Leu Phe Gly Leu Leu Leu Leu Phe Val Gly
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Val Gly Leu Phe Leu Leu Pro Ala Arg
3025 3030

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(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
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(10) International Publicati_n Number
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(21) International Application Number: PCT/US00/15527

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60/137,817 4 June 1999 (04.06.1999) US

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US 60/137,817 (CON)
Filed on 4 June 1999 (04.06.1999)

(71) Applicant (*for all designated States except US*): THE GOVERNMENT OF THE UNITED STATES OF AMERICA as represented by THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES [US/US]; Office of Technology Transfer, National Institutes of Health, Suite 325, 6011 Executive Boulevard, Rockville, MD 20852 (US).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): NAM, Jae-Hwan [KR/US]; 1001 Rockville Pike, #216, Rockville, MD 20852 (US). BUKH, Jens [DK/US]; 2018 Baltimore Road, #J42, Rockville, MD 20851 (US). EMERSON, Suzanne, U. [US/US]; 4517 Everett Street, Kensington, MD 20895 (US). PURCELL, Robert, H. [US/US]; 17517 White Ground Road, Boyds, MD 20841 (US).

(74) Agents: FEILER, William, S. et al.; Morgan & Finnegan, L.L.P., 345 Park Avenue, New York, NY 10154 (US).

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

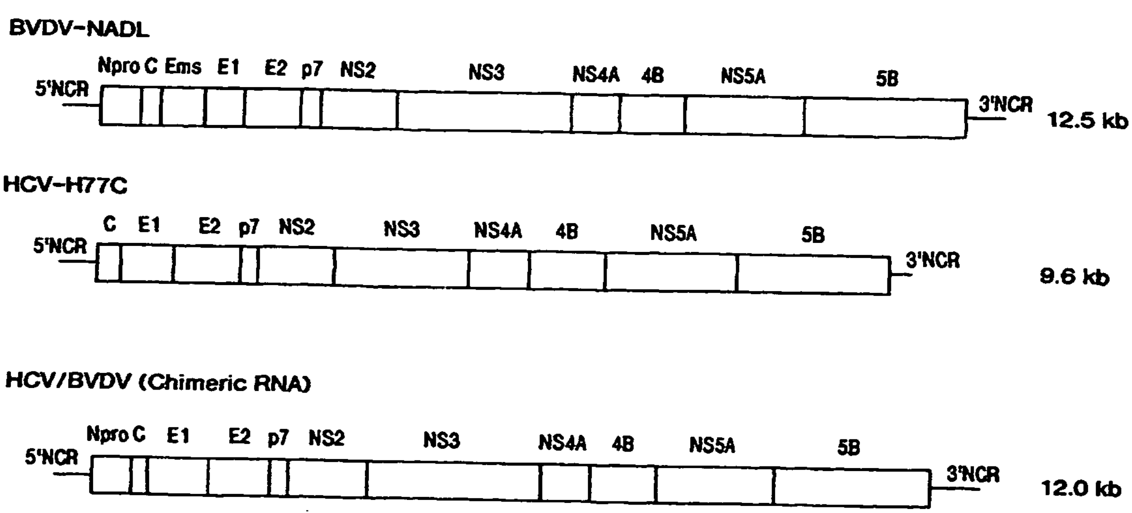
(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report: 15 November 2001

[Continued on next page]

(54) Title: HCV/BVDV CHIMERIC GENOMES AND USES THEREOF



(57) Abstract: The present invention relates to molecular approaches to the production of nucleic acid sequences which comprise the genomes of chimeric hepatitis C virus-bovine viral diarrhea viruses (HCV-BVDV). The invention also relates to the use of these chimeric nucleic acid sequences to produce chimeric virions in cells and the use of these chimeric virions in HCV antibody neutralization assays, and for the development of vaccines and therapeutics for HCV.

WO 00/75352 A3

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

Int ...tional Application No
PCT/US 00/15527

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/86 C12N7/01 C07K14/18

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, BIOSIS, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FROLOV I ET AL: "CIS-ACTING RNA ELEMENTS REQUIRED FOR REPLICATION OF BOVINE VIRAL DIARRHEA VIRUS-HEPATITIS C VIRUS 5' NONTRANSLATED REGION CHIMERAS" RNA,CAMBRIGDE UNIVERSITY PRESS, CAMBRIDGE,GB, vol. 4, no. 11, 25 November 1998 (1998-11-25), pages 1418-1435, XP000952790 ISSN: 1355-8382 the whole document ---	1,7,8, 11,12

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 February 2001	14. 02. 2001
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Chambonnet, F

2

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	H LU ET AL: "Poliovirus chimeras replicating under the translation control of genetic elements of HCV reveal unusual properties of the IRES of HCV" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA,NATIONAL ACADEMY OF SCIENCE. WASHINGTON,US, vol. 93, February 1996 (1996-02), pages 1412-1417, XP002919370 ISSN: 0027-8424 the whole document ---	12,13
X	VENUGOPAL K. & GOULD E.A.: "Towards a new generation of Flavivirus vaccines" VACCINE, vol. 2, no. 11, 1994, pages 966-975, XP002919372 GUILDFORD GB the whole document ---	11,12,20
P,X	WO 99 55366 A (FROLOV ILYA ;MCBRIDE M SCOTT (US); RICE CHARLES M (US); UNIV WASHI) 4 November 1999 (1999-11-04) page 4, line 21 - line 30 page 10, line 31 -page 11, line 17 page 11, line 33 -page 15, line 8; claims 1-10,16-21; figures 21,25,26; examples 1,2,4,5 ---	1,2,7,8, 14-21
A	MEYERS G ET AL: "RECOVERY OF CYTOPATHOGENIC AND NONCYTOPATHOGENIC BOVINE VIRAL DIARRHEA VIRUSES FROM CDNA CONSTRUCTS" JOURNAL OF VIROLOGY,THE AMERICAN SOCIETY FOR MICROBIOLOGY,US, vol. 70, no. 12, December 1996 (1996-12), pages 8606-8613, XP000952807 ISSN: 0022-538X the whole document ---	7
A	YU H ET AL: "SEQUENCE AND STRUCTURAL ELEMENTS AT THE 3' TERMINUS OF BOVINE VIRALDIARRHEA VIRUS GENOMIC RNA: FUNCTIONAL ROLE DURING RNA REPLICATION" JOURNAL OF VIROLOGY,THE AMERICAN SOCIETY FOR MICROBIOLOGY,US, vol. 73, no. 5, May 1999 (1999-05), pages 3638-3648, XP000946998 ISSN: 0022-538X the whole document --- -/--	7

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	LAI VC, ZHONG W, SKELTON A, INGRAVALLO P, VASSILEV V, DONIS RO, HONG Z, LAU JY: "Generation and characterization of a hepatitis C virus NS3 protease-dependent bovine viral diarrhea virus." JOURNAL OF VIROLOGY., vol. 74, no. 14, July 2000 (2000-07), pages 6339-6347, XP000952808 THE AMERICAN SOCIETY FOR MICROBIOLOGY., US ISSN: 0022-538X the whole document -----	7,8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/15527

B x I Observations wher certain claims w re found uns archabl (Continuation of it m 1 of first she t)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
   because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
   because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
   because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

B x II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [X] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

[X] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-6 11-13 partially 9, 10, 14-21

   A nucleic acid molecule comprising a chimeric virus genome, said genome being a BVDV genome in which the structural region of the BVDV genome has been replaced by the structural region of a hepatitis C virus genome; a DNA construct comprising said molecule; an RNA transcript of said DNA construct; a host cell transfected with said DNA construct or RNA transcript; a chimeric HCV-BVDV produced by said host cell; a composition comprising said virus.

2. Claims: 7, 8 and partially 9, 10, 14-21

   A nucleic acid molecule comprising a chimeric virus genome, said genome being a BVDV genome in which the non-structural region of the BVDV genome has been replaced by the non-structural region of a hepatitis C virus genome; a DNA construct comprising said molecule; an RNA transcript of said DNA construct; a host cell transfected with said DNA construct or RNA transcript; a chimeric HCV-BVDV produced by said host cell; a composition comprising said virus.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int tional Application No

PCT/US 00/15527

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9955366 A	04-11-1999	AU 3757199 A	16-11-1999
		EP 1071454 A	31-01-2001